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Toxicity Reduction Through Chemical and Biological Modification of Spent Pulp Bleaching Liquors

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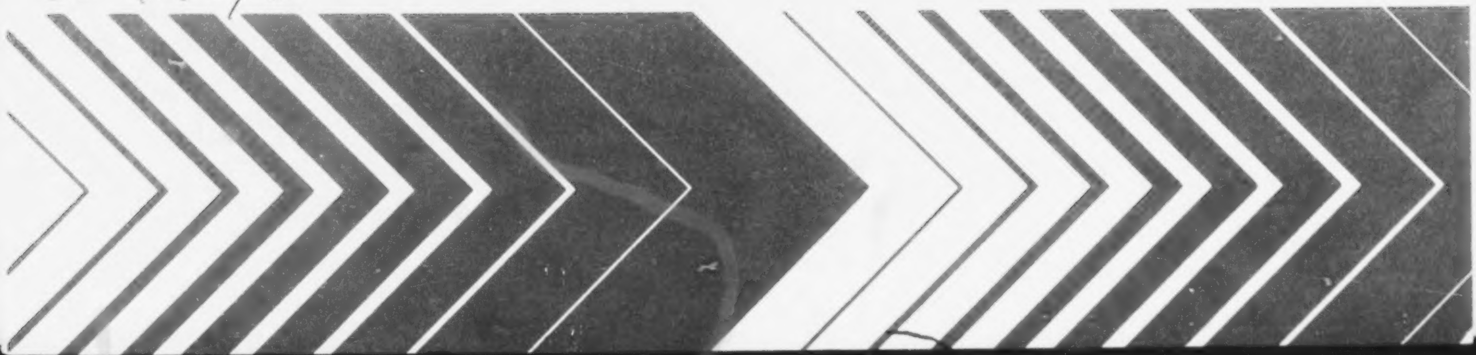

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January 1980

TOXICITY REDUCTION THROUGH CHEMICAL AND BIOLOGICAL MODIFICATION OF SPENT PULP BLEACHING LIQUORS

by

Carlton W. Dence, Chun-Juan Wang, and Patrick R. Durkin
State University of New York
College of Environmental Science and Forestry
Syracuse, New York 13210

Grant R 804779

Project Officers

Michael D. Strutz
H. Kirk Willard
Food and Wood Products Branch
Industrial Environmental Research Laboratory
Cincinnati, Ohio 45268

INDUSTRIAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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FOREWORD

When energy and material resources are extracted, processed, converted, and used, the related pollutional impacts on our environment and even on our health often require that new and increasingly more efficient pollution control methods be used. The Industrial Environmental Research Laboratory-Cincinnati (IERL-Ci) assists in developing and demonstrating new and improved methodologies that will meet these needs both efficiently and economically.

The report investigates the reduction of the toxicity of spent pulp bleaching liquors through chemical and biological modification. Toxicity was determined over a range of concentrations, before and after modification, to determine their effect on the growth of fungi, alga and duckweed, and on the survival of Daphnia Magna. Methods of chemical treatment that were investigated in this study included treatment with chlorine, chlorine dioxide, hypochlorite, ozonation and hydrogen peroxide treatment, alum and lime addition, carbon adsorption, and by modification of the conventional chlorination and caustic extraction bleaching stages. For further information on the subject, contact the Food and Wood Products Branch, IERL Cincinnati.

David G. Stephan
Director
Industrial Environmental Research Laboratory
Cincinnati

ABSTRACT

Chlorophenols similar to or identical with those detected in spent chlorination and caustic extraction liquors were synthesized and tested over a range of concentrations to determine their effect on the growth of several fungi, an alga (Chlorella pyrenoidosa), and duckweed (Lemna perpusilla) and on the survival of Daphnia magna. Although differences were found in the responses of the individual test organisms to the chlorophenols, the results generally indicated that growth repression and toxicity increased with increasing numbers of chloro substituents on the phenolic ring.

The chlorophenols referred to above were subjected to a number of chemical and biological treatments to assess their susceptibility to degradation when present as components of spent chlorination and caustic extraction liquors. Chlorophenol degradation was determined by gas chromatographic and/or ultraviolet spectroscopic analysis for unreacted starting material. Ozone and chlorine dioxide effectively degraded the chlorophenols, but complete removal required the application of excessively large amounts of chemical. In the case of the ozonization of tetrachloroguaiacol, decreases in phenol breakdown were paralleled by reductions in toxicity as indicated by Daphnia magna. Alkali treatment of the chlorocatechols at room temperature also proved an effective means of reducing chlorophenol toxicity.

Biological treatment of the chlorophenols consisted of the application of pure cultures of three different fungi and a mixed microbial population for periods ranging up to 15 days. Degradation varied widely among the various phenols and for the same phenol treated with different fungi. Under similar conditions, aeration in the absence of any microorganisms was effective in varying degrees with catechol (1,2-dihydroxybenzene) derivatives sustaining the greatest amount of degradation.

The spent liquors from the chlorination and caustic extraction bleaching stages of a pine kraft pulp (Kappa No. 25.5) were fractionated by means of dialysis, gel permeation chromatography, and solvent (ether) extraction; and the fractions bioassayed for their effect on a fungus, Aspergillus fumigatus and Daphnia magna. Without exception, the fraction containing the lower molecular weight materials displayed the greatest repression of fungal growth and were the most toxic to the Daphnia.

Spent chlorination and caustic extraction liquors were subjected to a variety of chemical treatments and the resulting effects on acute toxicity determined. Treatment with elemental chlorine, hypochlorous acid, hypochlorite, ozone and hydrogen peroxide produced increases in the toxicity of the spent liquor. A modest reduction in toxicity accompanied treatment of spent chlorination liquor with chlorine dioxide. Toxicity reduction was also achieved by the addition of alum and lime to spent caustic extraction liquor. The concentration of toxic material expressed as total organic carbon (TOC) was, however, higher in each instance. As in the case of the chlorophenols, substantial reductions of toxicity were achieved by the addition of alkali to spent chlorination liquor. Toxicity reduction in this instance paralleled decreases in phenol and organically bound chlorine contents.

Biological treatment of spent chlorination and caustic extraction liquors involved the application of a fungus (Candida utilis), an unidentified bacterium, and a mixed microbial population, together with supplemental carbon sources. The microbial mixture and the fungus effected essentially complete elimination of toxicity from spent chlorination liquor and small reductions in the toxicity of spent caustic extraction liquor. The bacterium was, on the other hand, comparatively ineffective in achieving the same objective.

Toxicity reduction through modification of conventional chlorination and caustic extraction bleaching stages was also evaluated. The substitution of chlorine dioxide for chlorine in the chlorination stage proved to be distinctly beneficial in reducing toxicity as was the substitution of oxygen for chlorine in the same stage. Small reductions in toxicity attended the introduction of hydrogen peroxide into the first caustic extraction stage but addition of hypochlorite in the same stage was less effective than alkali alone in this regard.

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SECTION 1

INTRODUCTION

It is now a generally accepted fact that bleaching effluents are mildly toxic to fish and other aquatic organisms. The results of a recent investigation (1) have shown that the compounds contributing to toxicity are resin acids, chlorinated resin acids, unsaturated fatty acids, fatty acid derivatives (epoxy stearic and dichlorostearic acid), and chlorophenolics. The presence of resin and fatty acids in bleaching effluents can be traced to inefficient washing of the bleached pulp. During the chlorination and caustic extraction stages, these compounds are in part converted to chloro and epoxy derivatives.

The chlorinated phenols are derived from the residual lignin in the pulp and appear to constitute the most toxic class of compounds comprising bleaching effluents. During chlorination, the chlorophenols are, to a large degree, oxidized and broken down to chlorine-substituted aliphatic compounds. The impact of the latter compounds on the toxicity of bleaching effluents has not been systematically evaluated.

Although the exact contribution of chlorophenols to the total toxicity of bleaching effluents may be difficult to assess with any degree of confidence at present, there can be little doubt but that it is at least a major and quite possibly dominant one. The continuing identification of chlorophenols in spent chlorination and caustic extraction liquors serves to emphasize the importance of learning how such compounds respond to various chemical and biological treatments in order that their removal from these spent liquors may be facilitated.

In response to the aforementioned need, the investigation described below was undertaken with the following objectives in mind:

1. To ascertain the relative degrees of toxicity displayed by phenols and chlorophenols similar to, or identical with, those previously detected in spent chlorination and caustic extraction liquors when contacted with a variety of aquatic life forms.

2. To determine the feasibility of using selected chemical and biological treatments or combinations of such treatments for the removal of those phenolic and chlorophenolic residues previously demonstrated to have a deleterious effect on aquatic life.

To pursue these objectives, we conducted experiments that involved acute toxicity bioassays, and biological and chemical treatments of the individual chlorophenols. These experiments were supplemented with similar tests that used the spent liquors from the chlorination and caustic extraction of a southern pine kraft pulp.

SECTION 2

CONCLUSIONS

Several species of fungi, an alga (Chlorella pyrenoidosa), and a vascular plant (Lemna perpusilla) were found suitable as plant test organisms for evaluating the toxic effects of phenols and chlorophenols. The sensitivity of these organisms decreased in the order alga > vascular plant > fungi. An aquatic invertebrate (Daphnia magna) also proved satisfactory for use in acute toxicity bioassays of the phenols and chlorophenols used in the investigation. In acute toxicity bioassays involving spent chlorination and caustic extraction liquors, Daphnia magna was found to be more useful than fungi due to its greater sensitivity.

Chlorophenols similar to or identical with those present in spent chlorination and caustic extraction liquors can be rapidly and extensively degraded with ozone and chlorine dioxide. However, their complete elimination requires inordinately large applications of chemical, particularly when present with other oxidizable materials as would be the situation in spent chlorination and caustic extraction liquors.

Alkaline treatment (pH 11) is effective in varying degrees in reducing the toxicity of solutions of the test chlorophenols. Chlorocatechols can be most successfully detoxified by such a treatment.

Chlorophenols of the type included in the investigation can be extensively degraded by simple aeration (in the absence of any microorganisms in 1-week treatments). A similar effect can be achieved by biological treatments involving individual fungi or a mixture of microorganisms. Fungi display widely varying degrees of ability to break down a given chlorophenol.

Some of the properties of spent chlorination and caustic extraction liquors were found to change as the liquors aged. Toxicity tests can be made on reasonably aged liquors, however, without the expectation of changes having occurred.

Based on the results of fractionation treatments, the toxicity of spent chlorination and caustic extraction liquors gives evidence of being attributable to the lower molecular weight substances contained therein.

In general, chemical treatment does not appear to represent a very promising approach for reducing or eliminating the toxicity of spent chlorination and caustic extraction liquors. However, detoxification of the former liquor through treatment with alkali may have a future, provided the technical and economic feasibility of such a treatment can be demonstrated by further experimentation. Lime and alum addition can be used to partially remove toxic material from spent caustic extraction liquor. However, the sludge that contains the toxic substances still has to be disposed of.

Modifications of conventional bleaching treatments (in which chlorine dioxide and oxygen are substituted for chlorine in the chlorination stage, and hydrogen perioxide is added to the caustic extraction stage) are effective in substantially reducing the toxicity of the corresponding effluents.

SECTION 3

RECOMMENDATIONS

As described below in the body of this report, attempts to reduce the toxicity of bleaching effluents through modification of conventional bleaching stages produced some very encouraging results. We recommend, therefore, that research along these lines be continued and expanded so that the potential of this approach may be fully assessed. In view of the general success the paper industry has had using biological forms of waste treatment, we further recommend that, in conjunction with the above bleaching stage modifications, the effluents from such treatments also be subjected to some form of biological treatment in order to assess the effect of the modification on their susceptibility to biodegradation.

Improved efficiency with respect to the biological systems used in the purification of paper mill wastes could result in an increased margin of safety with respect to the ability of a particular treatment system to consistently deliver toxicity-free effluents during mill upsets, or as a result of other operational problems. The wide range of abilities displayed by the various microorganisms used in the present investigation for breaking down chlorophenols suggests the possibility of manipulating the composition of the microbial population in the biological treatment system so as to facilitate this objective. On this basis, we recommend that the search for additional microorganisms having this particular capability be continued and that the effect of environmental test conditions (i.e., pH, nutrients, temp., etc.) be studied to ascertain how these should be controlled to achieve the maximum rate of degradation.

In the current investigation, acute toxicity bioassays relied largely on the response of a single species, Daphnia magna. This approach made it possible to screen a large number of phenols and chlorophenols as well as spent chlorination and caustic extraction liquors before and after various treatments. The relevance of this work could be substantially increased by applying more sophisticated test methods and by increasing the number of aquatic plant and animal species tested.

The feasibility of using an alga (Chlorella pyrenoidosa) and a vascular aquatic plant, duckweed, (Lemna perpusilla) as test organisms for acute toxicity bioassays of chlorophenols was explored in the present investigations and the findings (described in the body of this report) indicated their potential utility in this regard, particularly from the point of view of their increased sensitivity as compared to fungi. We propose that the tests involving these organisms be continued and broadened to include bleaching liquors before and after modification.

Although Daphnia represent an extremely important class of aquatic invertebrates and are thus a highly relevant test species, a sound program of hazard assessment should attempt to evaluate effects on several organisms and diverse habitats. For this reason, we recommend that bioassays of chlorophenols and spent chlorination and caustic extraction liquors be performed using Gammarus, a genus of amphipods commonly found in streams and brooks. Based on the comparative behavior of this organism and Daphnia when exposed to other toxicants, the use of Gammarus in acute toxicity bioassay might be expected to indicate significant differences in species susceptibility rather than simply parallel the results obtained using Daphnia.

SECTION 4

MATERIALS AND METHODS

CHEMICALS

2,4,6-Trichlorophenol, 2,4-dichlorophenol, catechol (1,2-dihydroxybenzene), guaiacol (2-methoxyphenol), p-cresol and phenol were obtained commercially. All other phenols and chlorophenols used in the investigation were prepared as described by Gess and Dence (2), Cain (3), Nonni (4), Dence et al. (5), and Willstätter and Müller (6).

Methyl dehydroabietate was prepared by methylation of the free acid with diazomethane. This ester was not isolated, but gas chromatographic analysis of the methylated material in the product mixture revealed the presence of only one peak.

BIOLOGICAL TEST ORGANISMS

Animals

Daphnia magna were purchased from Wards Natural Science Establishment, Inc. Stock cultures were maintained at 17 ± 1°C, pH 7.0 ± 0.1 units in DM_2, a reconstituted fresh water described by D'Agostino and Provasoli (7). The organisms were fed yeast and the photoperiod was kept on a 12-hour light/12-hour dark cycle. Algal and protozoal contamination of the stock cultures provided additional nutrient sources. First instar, or 1-3 day old *Daphnia*, for use in the biological assays were obtained by transferring groups of 25 nongravid mature organisms to 250 ml of fresh DM_2 (17 ± 1°C, pH 7.0) and adding 5 ml of homogenized yeast (1 mg/ml) per day until eggs developed. At this time, feeding was terminated and young were harvested daily.

Brine shrimp (*Artemia salina*) eggs, distributed by Metaframe Corporation, were purchased locally. These organisms were hatched in synthetic sea water (8) 24 hours prior to testing. The synthetic sea water was also used as the dilution water in all *Artemia salina* bioassays.

Plants

The plant test organisms used in the investigation consisted of several fungi (listed below in Table 1), an unidentified bacterial culture, an alga (*Chlorella pyrenoidosa* Chick), and a vascular plant (*Lemna perpusilla* Torr.), commonly known as duckweed.

TABLE 1. FUNGI USED IN THE INVESTIGATION

No.	Fungus name
1	Aspergillus fumigatus Fres. (C-78)
2	Cladosporium herbarum Link ex Fries (C-5)
3	Paecilomyces varioti Bainer (C-64)
4	Penicillium variabile Sopp (C-78)
5	Trichoderma koningii Oud. (C-65)
6	Aspergillus niger van Tiegh. (Wang 1152)
7	Candida utilis (Henneberg) Lodder & Kreger-van Rij

Fungi 1-5 were organisms most commonly isolated from samples collected at various sites of a kraft paper mill waste treatment plant. The bacterium included in the testing was identified in the same samples.

Candida utilis is an unicellar, yeast-like organism containing 50% edible protein.

SPENT BLEACHING LIQUORS

A pine kraft pulp (Kappa No. 25.5) was chlorinated and the resulting pulp extracted with alkali using the conditions described below:

	Chlorination	Caustic extraction
Chlorine or alkali applied, %	5.25	3.6
Residual chlorine, %*	0.1-0.2	-
Consistency, %	3	10
Time, min.	60	60
Temperature, °C	22	70
Final pH	1.7	12.3-12.4

*After filtration through a sintered glass Büchner funnel to remove the pulp fines, the residual chlorine was completely eliminated.

The chlorinated pulp was washed with dilute (0.01 N) HCl prior to the alkaline extraction stage in order to maintain the same pH environment in the pulp as existed during the chlorination.

FRACTIONATED SPENT BLEACHING LIQUORS

Dialysis

Spent chlorination liquor was dialyzed in a cellophane tube against distilled water for 24 hours after first being concentrated to 1/10 of its original volume. The ratio of spent liquor to distilled water during dialysis was 1:40. Spent caustic extraction liquor was dialyzed directly under the same conditions. In the case of extensive dialysis of the spent caustic extraction liquor, the dialysis was repeated after the dialyzed liquor was concentrated to its original volume. When sodium ion removal was required, the retentate was further dialyzed against 0.1 N HCl and then against distilled water.

Gel Permeation Chromatography

A concentrated neutralized sample of spent liquor (10 ml) was charged onto the top of a 20 mm x 1 mm column of Sephadex G-25 (40 g) and developed by elution with distilled water. The elution of material was followed by measuring the absorbance of the eluate at 280 nm.

Ether Extraction

Three liquid-liquid extractors (400 ml, 1-ℓ and 10-ℓ capacity) were used depending on the experimental requirements. Spent chlorination liquor was extracted directly; spent caustic extraction liquor was extracted after acidifying to pH 2 and without removing the precipitate which thereupon formed.

Silica Gel Chromatography

Ether extracts of the spent liquor were further fractionated on a column of 60-200 mesh silica gel (Grade 950, Fisher Chemical Company). A layer of anhydrous sodium sulfate was added to the silica gel to remove small amounts of water in the solvent extract. The ratios of sample to silica gel and sodium sulfate were 1:150 and 1:25, respectively. The column was eluted consecutively with petroleum ether, diethyl ether, and methanol.

CHEMICAL TREATMENT OF CHLOROPHENOLS

Chlorine Dioxide

Ten millimoles of the phenols in 30-40% aqueous ethanol were reacted in the dark at room temperature with amounts of chlorine dioxide ranging from 1-15 equivalents/mole of phenol until the oxidant was completely exhausted. The product mixtures were exhaustively extracted with chloroform to remove any unreacted phenol and the extracts were dried over anhydrous $MgSO_4$, concentrated to ∿3 ml, and subjected to gas chromatographic analysis. Prior to gas chromatographic analysis, the extract containing the one catechol tested was silylated as described in the section Gas Chromatographic Analysis.

In an experiment designed to test the efficiency of phenol oxidation in the presence of a bleaching liquor, a solution of tetrachloroguaiacol (24 mg) was diluted with varying amounts of spent caustic extraction liquor and reacted with 13 mg of chlorine dioxide at room temperature until the latter was depleted. The residual phenol was recovered by extraction with chloroform and determined by gas chromatographic analysis as described above.

Ozone

Ozone was generated with a Welsbach Model LOA-1 PSI corona generator using oxygen as the gas source. Two 1-liter gas washing bottles connected in series were attached to the generator. Both bottles were equipped with fritted glass diffuser tubes to provide a small bubble size. The rate of ozone generation was determined by placing a 5% KI solution in one of the bottles and measuring the amount of iodine liberated in a given time interval following introduction of the ozone stream.

The phenols (0.207 mmole) were each dissolved in 500 ml of a 20% ethanol (v/v) solution containing 3 g of a buffer mixture comprised of 90 g of KH_2PO_4 and 8 g of K_2HPO_4. This buffer concentration produced an initial pH of approximately 6.15 which stayed virtually constant throughout the reaction period. The phenol solutions were ozonized at ambient temperature (22 ± 2°C) for 30-second intervals initially and for longer periods near the end of the treatment. After each reaction interval, the ozone was diverted to the atmosphere through a t-joint in the assembly and a 3-ml sample was withdrawn from the reactor and analyzed by ionization difference spectroscopy (9).

As a check on the accuracy of the above method, the residual phenol content of a few ozonized solutions was measured by gas chromatography. In these tests, 200 ml of a 4.14×10^{-4} M solution of each model (in 20% ethanol) containing 1.2 g of the same buffer mixture described above was divided into two equal portions. One 100-ml portion was set aside as a control and the 100-ml portion was reacted with ozone for one minute at a flow rate of either 2.64 or 3.6 mg/min. At the conclusion of the reaction, both the control and ozonized samples were extracted with 70 ml of chloroform. The solvent phase was dried over anhydrous $MgSO_4$, evaporated to ∿3 ml and silylated.

In the treatment of fully etherified phenols, a new 500-ml sample was used for each ozonization reaction period. The ozonized product mixtures in this instance were extracted with 250 ml of chloroform. The extracts were dried over anhydrous $MgSO_4$, concentrated to ∿3 ml, and analyzed by gas chromatography.

Tetrachloroguaiacol was ozonized in an alkaline medium. A series of solutions was prepared, each member of which contained a 0.1 mmole amount of this phenol dissolved in 100 ml of a buffer solution 0.08 N in Na_2CO_3 and 0.02 N in $NaHCO_3$ (pH 10.3). These solutions were reacted at a flow rate ranging from 0.6 to 0.8 mg/min. At the end of this treatment, the product mixtures were acidified and extracted with ether. The solvent layers were dried over anhydrous Na_2SO_4, concentrated to dryness, and the residues redissolved in $CHCl_3$ prior to gas chromatographic analysis.

CHEMICAL TREATMENT OF SPENT CHLORINATION AND CAUSTIC EXTRACTION LIQUORS

Chlorine-Containing Oxidants

Elemental chlorine (i.e., chlorine water), hypochlorous acid, and chlorine dioxide were applied to spent chlorination liquor and allowed to react to complete exhaustion at room temperature. In the application of hypochlorous acid, the reaction mixture was buffered to pH 4.75 by the addition of acetate. Sodium hypochlorite was applied to spent caustic extraction liquor at 60°C and the reaction was continued until the oxidant was completely consumed.

Ozone and Hydrogen Peroxide

All ozone treatments of the spent liquors were performed at room temperature using the ozone generator described above. A 250-ml sample of spent chlorination liquor (pH 2) was reacted with ozone introduced at a flow rate of 4.65 mg/min. The first 9.3 mg of ozone applied were completely consumed by the liquor under the test conditions. The pH of a 250-ml sample of spent chlorination liquor was buffered at 8.0 by the addition of 4 g of K_2HPO_4. The ozone flow rate was 4.75 mg/min. and the first 16.6 mg of ozone were completely absorbed by the liquor. A 150-ml sample of spent caustic extraction liquor was reacted with ozone (flow rate, 4.75 mg/min.) after the pH was first adjusted to 8.0 by the addition of 2 g of KH_2PO_4. Absorption of ozone was complete up to the addition of 143 mg ($\sim$30 min.).

Spent caustic extraction liquor was buffered ($\sim$pH 10.5), sodium silicate and magnesium sulfate added, and the mixture reacted with hydrogen peroxide at 70°C. Consumption of peroxide occurred slowly and not until after 24 hours was the applied dosage (4 meq/150 ml of liquor) totally consumed.

Sodium Hydroxide

The pH of spent chlorination liquor was adjusted to various levels by the addition of sodium hydroxide and maintained at the initial level by the addition of more alkali as required. After a predetermined reaction period at room temperature, the phenol and organically bound chlorine contents and the acute toxicity of the solutions were determined, as described in later sections.

Aluminum Sulfate, Lime and Activated Carbon

Aluminum sulfate (1.5 g) was added to spent caustic extraction liquor (220 ml) and allowed to react for one hour at room temperature (pH 5.5). The precipitate was removed by centrifugation, dissolved by addition of HCl, and subsequently dialyzed through cellophane. The supernate from the centrifugation and the retentate from the dialysis were diluted to the volume of the original test sample, tested for phenol content, and bioassayed for acute toxicity.

Lime treatment of spent caustic extraction liquor consisted of the addition of a saturated solution of slaked lime ($Ca(OH)_2$) containing 2.2 g of the latter to a 220-ml sample of the liquor (pH 11). The reaction mixture was stirred for several hours at room temperature and the precipitate removed by centrifugation. Carbon dioxide was introduced into the supernate to precipitate the calcium as $CaCO_3$. The combined precipitates were redissolved by the addition of HCl and dialyzed through cellophane for two days with frequent changes of water. The volumes of supernate and dialysis retentate were adjusted to the original sample volume and the resulting solutions analyzed for phenol content and acute toxicity.

In those instances where spent caustic extraction liquor was treated sequentially with lime and activated carbon or a chemical oxidant such as chlorine dioxide, ozone or hydrogen peroxide, a solution of slaked lime (15 g/l) was added to the liquor; the mixture stirred for 20 minutes, and then allowed to stand for one hour. The precipitate was removed by filtration through No. 41 Whatman filter paper. Subsequent treatment of the filtrate was proceeded by pH adjustment to 9.4 by the addition of carbon dioxide and to 5.0 with HCl. The $CaCO_3$ formed after CO_2 addition was removed by filtration.

Treatments of spent caustic extraction liquor with activated carbon were performed using a sample of Nuchar S-A provided by Westvaco Corporation. Samples of the liquor were treated in a batch process with the activated carbon for 18 hours with continuous stirring. At the end of this period, the carbon was removed by filtration through No. 41 Whatman paper.

CHLORINATION AND CAUSTIC EXTRACTION STAGE MODIFICATIONS

In the sequential ClO_2/Cl_2 treatment, 1.0% of ClO_2 and 2.6% of Cl_2 were applied to the kraft pulp at room temperature and a consistency of 3%. These combined amounts were equivalent to 5.25% chlorine and the proportions corresponded to a 50% replacement of chlorine with chlorine dioxide. The bleaching was continued until the chlorine was exhausted (final pH, 2.1). Chlorine dioxide (2%) alone was applied to the pulp under the same conditions used for the sequential treatment (final pH, 2.85).

Peroxide treatment of a chlorinated kraft pulp consisted of applying 0.5% H_2O_2 (10% cons., 70°C, 3 hr) after first adjusting the pH of the bleach to 11.2. Hypochlorite treatment as a replacement of the conventional caustic extraction stage consisted of applying 2% of sodium hypochlorite to the pulp (10% cons., 45°C, 1.5 hr, initial pH $\sim$12.5). In each case, the oxidant was consumed to complete exhaustion at the conclusion of the reaction period.

The alkali/oxygen liquor was prepared by treatment of a sample of southern pine kraft (Kappa No. 32.9) with 2.5% of oxidized white liquor (as NaOH) at 80 psig oxygen at 27% consistency for 30 minutes at 110°C. The liquor used in the acute toxicity bioassay was obtained by diluting the 27% consistency pulp to 4% and centrifuging off the liquor. The total organic carbon (TOC) content of the liquor was 405 ppm.

BIOLOGICAL TREATMENT OF PHENOLS AND CHLOROPHENOLS

Aliquots of stock solutions of the various phenols (3 mg/ml in 40% ethanol) were chosen such that after dilution to the final volume (100 ml), the concentrations of compounds having one or less chloro substituents and those having two or more chloro groups were 10 and 50 ppm, respectively. One ml of each fungal spore suspension (10) was used as inoculum. For each of the phenols tested, a control sample containing no fungus was set up and inoculated with sterile distilled water. Two replicates were prepared at each concentration level and also for the control.

Flasks containing the solutions described above were placed on a horizontal shaker and incubated at 28°C for one week. Following the incubation period, the contents of each flask were filtered through a Seitz filter and the filtrates of replicate tests combined to yield approximately 200 ml of solution. Identical volumes of the filtrates were extracted with chloroform, and the latter was concentrated _in vacuo_ to 3 ml and dried over anhydrous magnesium sulfate. The dried solvent extract was subsequently analyzed for residual phenol by gas chromatography as described in a later section.

BIOLOGICAL TREATMENT OF SPENT CHLORINATION AND CAUSTIC EXTRACTION LIQUORS

The pH of both the spent chlorination liquor (SCL) and spent caustic extraction liquor (SCEL) were adjusted to 7.0 with sodium hydroxide and hydrochloric acid, respectively, over a two-hour period. Samples of the liquors were sterilized by filtration through a Seitz filter fitted with a #6 sterilizing pad. One hundred-ml aliquots of the filtrates were subsequently transferred to sterile 250-ml Erlenmeyer flasks. One sample of each liquor was inoculated with 5.0 ml of sludge (obtained from a bleached kraft mill waste treatment plant), yeast or bacterial cell suspension. In addition, one flask was left as an uninoculated control. The flasks were placed on a horizontal shaker and incubated at 28°C for 7 days. Following the incubation period, the flasks were filtered using a Seitz filter fitted with a sterilizing pad. The filtrates of both the test and control samples were then bioassayed for acute toxicity using _Daphnia magna_ as the test organism. The preparation of the fungal and bacterial inocula and the composition of the buffer and nutrient solutions are individually described below for the treatment with sludge (mixed microbial population) yeast (_Candida utilis_) and bacterium.

Sludge (mixed microbial population

In the initial experiment, asparagine, at a concentration of 0.2 g/l, was added as a nitrogen source and monobasic potassium phosphate at a concentration of 0.01 g/l, served as a phosphorous source. A second experiment was conducted in which no supplemental nutrients were added to the liquors. In this experiment, monobasic potassium phosphate (0.10 g/l) was added to the spent caustic extraction liquor (SCEL) and dibasic potassium phosphate (0.10 g/l) was added to the spent chlorination liquor (SCL).

Yeast (*Candida utilis*)

Monobasic potassium phosphate (0.10 g/l) was added to the spent caustic extraction liquor and dibasic potassium phosphate (0.10 g/l) was added to the spent chlorination liquor. Preliminary experiments showed that *C. utilis* could not grow in spent bleaching liquors in which no supplemental nutrients were added. Therefore, glucose at a concentration of 1.0 g/l was added to the liquors.

Inoculum was prepared according to the following procedure: Several loopfuls of a 3-day-old culture of *C. utilis* (NRRL Y-900) growing on yeast morphology slants were dispersed in 50 ml of sterile distilled water in a 125-ml Erlenmeyer flask. Enough yeast cells were added to yield a cell suspension having an absorbance reading of 0.32 at 540 nm measured on a Spectronic-20 spectrophotometer.

A Bacterium

Mono and dibasic potassium phosphate were added to samples of the filter-sterilized SCEL and SCL, respectively, at a concentration of 1.0 g/l. In addition, glucose at a concentration of 1.0 g/l was added to both liquors.

Inoculum was prepared as follows: Several loops of a 4-day-old bacterial culture (Ziobro, culture 2-B) growing on nutrient agar slants were dispersed in 20 ml of sterile distilled water. Enough cells were added to yield a cell suspension having an absorbance reading of 0.45 at 540 nm, measured on a Spectronic-20 spectrophotometer.

ACUTE TOXICITY TESTS

Daphnia magna

Acute toxicity tests were conducted on the various materials using either first instar or 1-3 day old animals. The dilution water used in the tests was DM_2 prepared from doubly distilled water (5). This solution had a pH of 7.0 and was aerated to saturation prior to use. Stock solutions of the various test compounds were prepared by dissolving the appropriate amount of the material in a suitable solvent (DMSO) and adding the desired volume of DMSO/toxicant solution to the dilution water. Spent liquor samples were first adjusted to pH 7.0-7.5 through the addition of NaOH or HCl immediately prior to initiating the test. The toxicant solutions were then serially diluted to the required concentration levels. Each test consisted of 5-6 concentration levels with dilution factors of 0.5-0.9 between each concentration and 10 organisms per concentration. All tests were conducted at 17 ± 1°C in a constant temperature bath. Lack of gross movement was used as the criterion for death. Concentration-response patterns were either subjected to probit analysis (11) using the Bio-Med computer at Syracuse University or through the use of tables (12,13) based on the moving average method of Thompson (14).

Brine Shrimp

Before initiating each assay, the spent liquors were adjusted to pH 7.4-7.6 with either NaOH or HCl and the salinity was adjusted to that of the standard sea water formulation (22 parts/thousand) by adding NaCl. The organisms were exposed to test volumes of 50 ml in Carolina culture dishes having dimensions of 3.5 x 1.5 inches. Each liquor was tested at five concentrations with 20 organisms per concentration and a dilution factor of 0.5 between concentrations. During the tests, the pH ranged from 7.2 to 7.6 and temperature ranged from 20 to 22°C. Mortality was recorded after 24 hours and mortality-response patterns were subjected to probit analysis (11).

Fungi

The basal medium for the bioassy consisted of a solution of 3.00 g of glucose, 1.25 g of peptone, and 1.25 g of yeast extract in 1000 ml of distilled water (pH 6.5). Each test compound was dissolved in 40% ethanol and maintained at a stock concentration of 3.0 mg/l (3000 ppm). The stock solution was serially diluted to concentration levels of either 200, 100, 50, 10 and 0 ppm (control) or 50, 25, 10, 7.5 and 0 ppm (control), depending upon the number of chlorine atoms in each compound. After dilution of the test solution with the basal medium and addition of the spore suspension, the final volume was 20 ml and the concentration of ethanol, 2%.

Five replicate samples of eight phenols were prepared for assay of _Aspergillus fumigatus_. Two replicate samples of 17 phenols were tested against the six additional species of fungi used in the investigation. The test solutions were placed on a horizontal shaker and incubated at 28°C for 3 days. Growth was measured by visual observation and by dry weight determination of the fungal mycelia.

Acute toxicity bioassays of spent chlorination and caustic extraction liquors were also performed, using six common fungi as the test organisms. Prior to testing, the pH of the two liquors was adjusted to near neutrality by the addition of NaOH or HCl. Duplicate samples of each of the whole liquors and samples diluted to 1/2, 1/4 and 1/10 of their initial volumes were added to a glucose-peptone-yeast medium and one ml of the various spore suspensions added to each. The samples were placed on a horizontal shaker and incubated for 7 days at 28°C. Growth of the various cultures was estimated visually.

Alga

The green alga, Chlorella pyrenoidosa, was grown in modified Jack Myer's medium for Chlorella and Scenedesmus in acid-clean flasks on a rotary shaker (150 rpm) at 18°C and under an illumination of 650 foot-candles of continuous light. Inoculum was prepared as described in the EPA publication, "Algal Assay Procedure: Bottle Test" (15), except that a larger amount of inoculum was used (10^5 cells/ml). Test chemicals were dissolved in ethanol and the final concentration of ethanol in the culture medium was 0.1%. The absorbance of the alga in the growth medium, measured spectrophotometrically at 650 nm, was used as a measure of the amount of growth.

The initial concentrations of the phenols tested were 100, 10, 1, and 0.1 ppm or 10, 1, 0.1, and 0.01 ppm depending on the anticipated degree of toxicity. The range of toxicity was determined to be from the level of concentration at which no growth occurred to that where some growth occurred, even if it was reduced from that of the control. Toxicity levels were delineated more precisely by selecting and testing a variety of concentrations from within the previously determined toxicity range. The absorbance of the alga in the growth medium (measured spectrophotometrically at 650 nm) was used as a measure of the amount of growth at the end of 12 to 14 days.

Toxicity tests of spent chlorination and caustic extraction liquors were performed in a manner similar to those described above for the phenols with the following modifications: The liquors were with NaOH or HCl, buffered with phosphate and sterilized with a Seitz filter prior to being tested. The intense color of the liquors prevented effective use of spectrophotometry to record the results. As an alternative approach, direct counts from a hemocytometer were used. Liquor dilutions of 1:2, 1:4 and 1:8 were tested.

Duckweed

One half strength of Hutner's medium plus sucrose was used for growing Lemna perpusilla (duckweed). The test phenols were dissolved in 4% DMSO. 2,4-Dichlorophenol, and 4,5-dichloroguaiacol were tested at concentrations of 20, 15, 10 and 5 ppm. 4,5-Dichlorocatechol and 2,4,6-trichlorophenol were tested at concentrations of 10, 5, 1, and 0.1 ppm. Each flask containing 50 ml of the culture medium and the phenol solution was inoculated with a 3-frond colony of L. perpusilla. The flasks were incubated at 25°C under a constant illumination of 150 foot-candles for 10 days. The number of fronds was counted using a stereoscopic microscope.

GAS CHROMATOGRAPHIC ANALYSIS

Dehydroabietic Acid

Pine kraft pulp (Kappa No. 25.5) was washed with water, dewatered to 36.5% consistency and extracted consecutively for 24-hour periods with 8000-, 5000-, and 5000-ml amounts of sodium hydroxide solution (pH 10.5) at room temperature. After each treatment, the pulp was filtered and washed with 5 x 500 ml of water. The three filtrates were concentrated separately to ∿2500 ml, acidified to pH 2 and extracted with $CHCl_3$.

The chloroform extracts were reacted with CH_2N_2 to convert any carboxylic acids contained therein to their corresponding methyl esters. The derivatized esters were then subjected to gas chromatographic analysis using an F and M Model 720 gas chromatograph fitted with a 40" x 1/4" O.D. stainless steel column filled with 3% SE-30 on 80-100 mesh acid-washed, silylated Chromosorb G. The identification of methyl dehydroabietate was made on the basis of spiking with the authentic ester. The yield of the ester was determined by comparison of its gas chromatographic peak height with that corresponding to a standard.

In another experiment, a sample of the kraft pulp was extracted with a sodium hydroxide solution (pH 12.4) for 60 minutes at 70°C. The pulp was filtered, washed with water, and re-extracted under the same conditions. The filtrates from the two extractions were concentrated separately, acidified to pH 2, and extracted with chloroform. The chloroform extracts were derivatized and analyzed gas chromatographically for methyl dehydroabietate as described above.

Phenols and Chlorophenols

Gas chromatographic analysis of the phenols and chlorophenols in the various product mixtures was performed using either an F and M Model 720 (thermal conductivity detector) or a Varian Aerograph Model 1700 (flame ionization detector) instrument. In all cases, separations were achieved using stainless steel or aluminum columns containing 3% SE-30 on 80-100 mesh acid-washed, silylated Chromosorb G. The columns were heated, both in the isothermal and programmed modes, and the temperatures ranged from 150-250°C.

Monohydric phenols and guaiacol (2-methoxyphenol) derivatives were chromatographed without derivatization. Catechol (1,2-dihydroxybenzene) derivatives were, on the other hand, converted to di-trimethylsilyl ethers prior to injection by reaction with an excess of bis(trimethylsilyl) acetamide ("BSA"). Quantitative estimation of the amounts of phenols was made by comparison of peak height or area with those of standards.

ANALYTICAL PROCEDURES

BOD_5 analysis was performed according to the procedure outlined in "Standard Methods for the Examination of Water and Wastewater" (16). Total organic carbon (TOC) measurements were performed on a Beckman Model 915 Total Organic Carbon Analyzer. Color measurements were based on the cobalt-platinum scale described by Brown (17).

Acidic groups were measured by dissolving the sample in a pyridine-water-butanol mixture and titrating with alcoholic sodium hydroxide using a pH meter fitted with a glass-calomel electrode system. Total hydroxyl content was estimated using the procedure described by Siggia (18) modified by performing the acetylation at room temperature for 17 hours.

Molecular weights were determined using a Hewlett-Packard Model 302 vapor pressure osmometer. A non-aqueous probe thermostatted at 65°C was used and the samples were dissolved in methyl cellosolve. Benzil was used as a calibration standard.

Chloride was measured by potentiometric titration with standard $AgNO_3$ solution as described by Shiner and Smith (19) and modified by Braddon and Dence (20). Organically bound chlorine was determined by applying an appropriate size sample (adjusted to pH 8 if not initially alkaline) on a 5.5 cm No. 1 Whatman filter paper while simultaneously evaporating the water with the aid of a hair dryer. The dried filter paper was burned in a Schoniger combustion flask containing 50 ml of distilled water. Potentiometric titration of the resulting solution with standard $AgNO_3$ solution provided the total chlorine (inorganic and organic) in the sample. The organically bound chlorine was calculated by subtraction of the inorganic chloride content (see above) of the original sample from this value.

Chlorine, chlorine dioxide, ozone and hydrogen peroxide were determined iodometrically. In the case of hydrogen peroxide, the reduction was catalyzed by the addition of ammonium molybdate.

Ionization difference spectra were recorded on a Unicam Model SP800A recording spectrophotometer. Semimicro matched quartz cells having either a 1- or 10-mm light path were used.

SECTION 5

RESULTS AND DISCUSSION

ACUTE TOXICITY OF PHENOLS

The phenols and phenol ethers used in the investigation are listed in Table 2. Their selection was based on a number of criteria, including mainly structural identity or similarity to compounds previously identified in spent chlorination and caustic extraction liquors and a desire to achieve a broader understanding of the structure-toxicity relationships pertaining specifically to these types of phenols and chlorophenols. The phenols listed in Table 2 thus consist principally of the various chlorine-substituted derivatives of mono- and dihydric (i.e., catechol) ring types. Fully methylated structures were utilized only to a limited degree and that solely for the purpose of gauging the effect of a free phenolic hydroxyl group in a particular measurement or treatment.

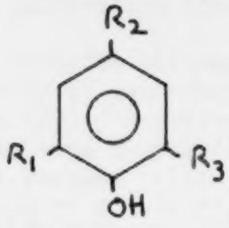
In the initial phase of the project, the phenolic compounds studied contained a methyl substituent at the position para to a phenolic hydroxyl group (i.e., at the side chain position). Later, these compounds were abandoned in favor of structures containing a chloro substituent at the para position since the latter types were concurrently being identified in spent chlorination and caustic extraction liquors (21-23). The general behavior of the two aforementioned structural types with respect to reactivity and acute toxicity probably do not differ greatly, especially when a comparison is made between compounds having identical numbers of chloro substituents on the same type of phenolic ring.

Effect on Fungi

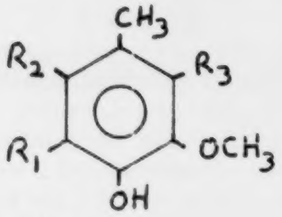
A detailed study was made of the effect of eight phenols on *Aspergillus fumigatus*. The plots shown in Figures 1 and 2 indicate the effect of increasing concentrations of the phenols on the amount of fungal growth. These plots reveal that inhibition of fungal growth increased in proportion to the number of chloro substituents attached to the phenolic ring. 2,4,6-Trichlorophenol (VI, Table 2) had the greatest effect of the phenols tested in reducing the growth of the fungus.

Expansion of the testing to include five additional fungi and a total of 17 phenols produced the results shown in Table 3. Each of the fungi used in the testing had been previously found to be present in water sampled at various sites of a bleached kraft mill waste treatment plant. Although

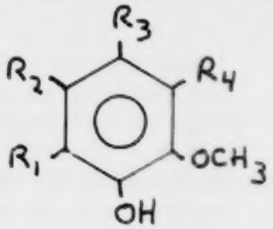
TABLE 2. PHENOLIC COMPOUNDS USED IN THE INVESTIGATION



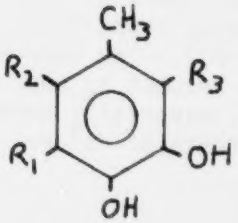
I $R_1=R_2=R_3=H$
II $R_1=R_3=H;\ R_2=CH_3$
II $R_1=R_3=H;\ R_2=Cl$
IV $R_1=R_2=Cl;\ R_3=H$
V $R_1=R_3=Cl;\ R_2=CH_3$
VI $R_1=R_3=R_3=Cl$


CH3
R2
R3
R1
OCH3
OH

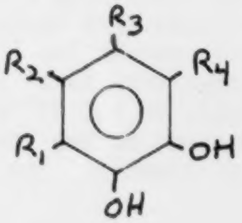
VII $R_1=R_2=R_3=H$
VIII $R_1=Cl;\ R_2=R_3=H$
IX $R_1=R_3=H;\ R_2=Cl$
X $R_1=R_2=Cl;\ R_3=H$
XI $R_1=R_3=Cl;\ R_2=H$
XII $R_1=H;\ R_2=R_3=Cl$
XIII $R_1=R_2=R_3=Cl$


R3
R2
R4
R1
OCH3
OH

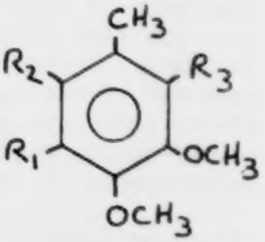
XIV $R_1=R_4=H;\ R_2=R_3=Cl$
XV $R_1=R_2=R_3=Cl;\ R_4=H$
XVI $R_1=R_2=R_3=R_4=Cl$


CH3
R2
R3
R1
OH
OH

XVII $R_1=R_2=R_3=H$
XVIII $R_1=R_3=H;\ R_2=Cl$
XIX $R_1=R_2=Cl;\ R_3=H$
XX $R_1=R_2=R_3=Cl$


R3
R2
R4
R1
OH
OH

XXI $R_1=R_4=H;\ R_2=R_3=Cl$
XXII $R_1=R_2=R_3=Cl;\ R_4=H$
XXIII $R_1=R_2=R_3=R_4=Cl$


CH3
R2
R3
R1
OCH3
OCH3

XXIV $R_1=R_2=R_3=H$
XXV $R_1=Cl;\ R_2=R_3=H$
XXVI $R_1=H;\ R_2=R_3=Cl$
XXVII $R_1=R_2=R_3=Cl$

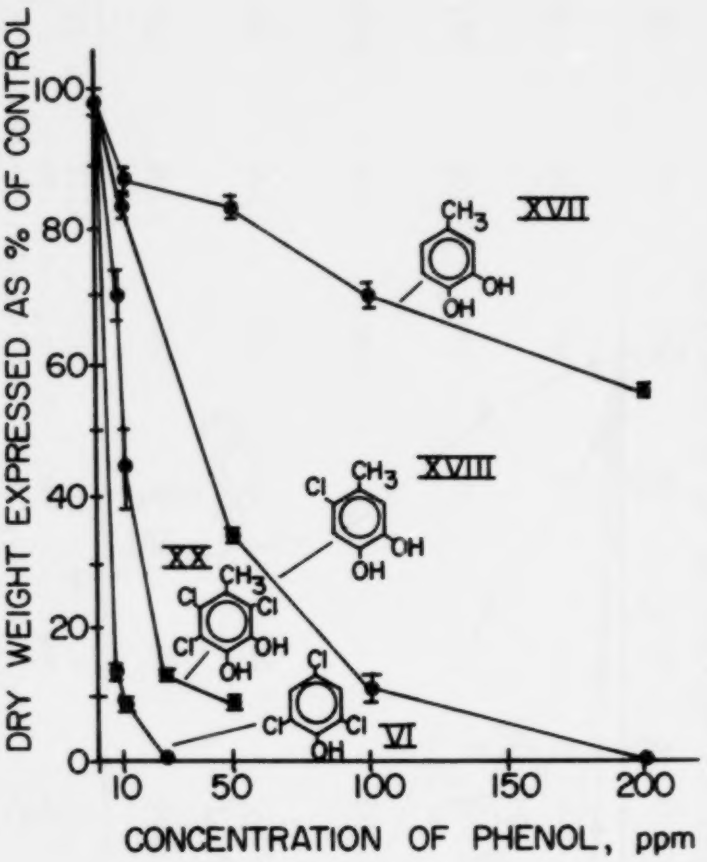


Figure 1. Effect of 2,4,6-trichlorophenol (VI) and three chlorocatechols on the growth of Aspergillus fumigatus. (Values for dry weight are based on five replicates. Vertical bars represent the standard error of the replicates.)

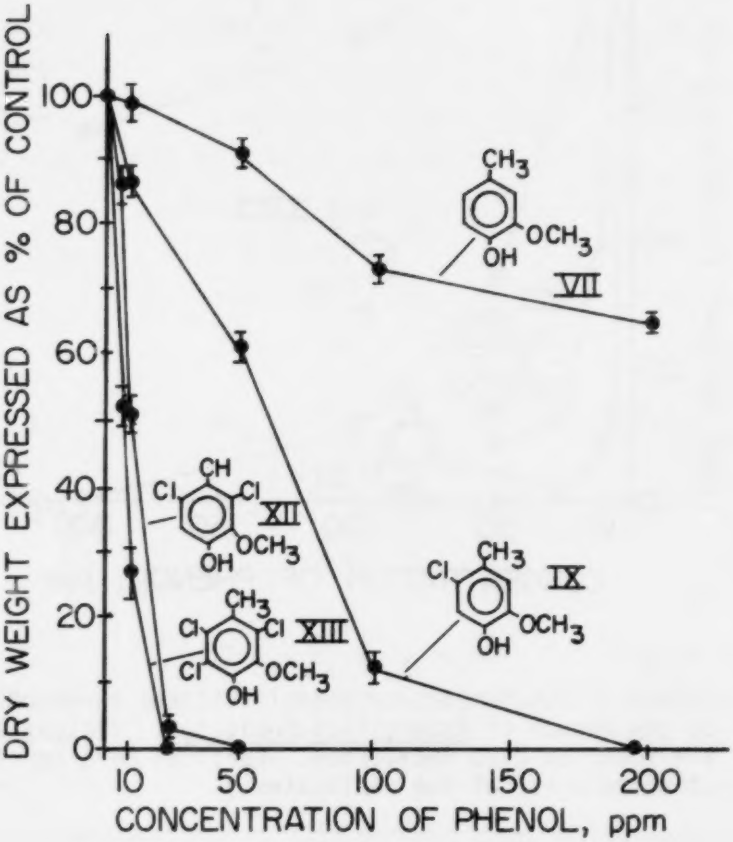
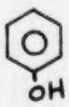
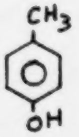
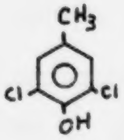
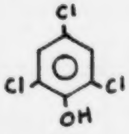
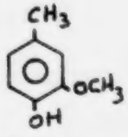
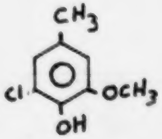
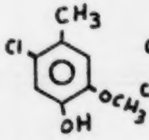
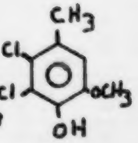


Figure 2. Effect of creosol (VII) and three chloroguaiacols on the growth of Aspergillus fumigatus. (Values for dry weight are based on five replicates. Vertical bars represent the standard error of the replicates.)

TABLE 3. MINIMUM CONCENTRATIONS (IN PPM) OF PHENOLS REQUIRED TO PREVENT GROWTH OF VARIOUS FUNGI

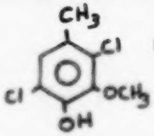
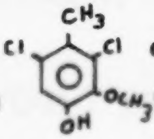
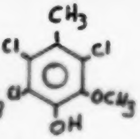
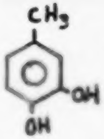
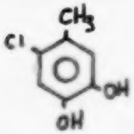
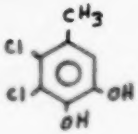
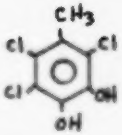
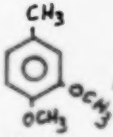
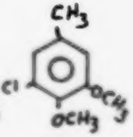
Phenol Fungus	 I	 II	 V	 VI	 VII	 VIII	 IX	 X
Trichoderma koningii	>[b]200	>200	25	25	>200	100	100	25
Aspergillus niger	> 200	>200	50	10	>200	100	200	25
Aspergillus fumigatus	> 200	>200	25	10	>200	100	200	25
Paecilomyces varioti	> 200	>200	50	10	>200	100	200	50
Cladosporium herbarum	> 200	>200	25	7.5	>200	50	50	25
Penicillium variabile	> 200	>200	50	25	>200	200	100	25

[a] In ppm

[b] >Indicates growth was evident at the highest concentration tested.

(continued)

TABLE 3 (continued)

Phenol Fungus	 XI	 XII	 XIII	 XVII	 XVIII	 XIX	 XX	 XXIV	 XXV
Trichoderma koningii	>[b]50	25	>50	100	100	>50	50	>200	200
Aspergillus niger	> 50	25	25	>200	200	>50	50	>200	>200
Aspergillus fumigatus	> 50	25	25	>200	200	>50	25	>200	>200
Paecilomyces varioti	50	25	25	>200	200	>50	25	>200	>200
Cladosporium herbarum	25	25	10	100	100	25	7.5	100	100
Penicillium variabile	> 50	50	>50	>200	100	>50	25	>200	>200

b >Indicates growth was evident at the highest concentration tested.

some variations occurred in the response of the isolates, the findings generally duplicated those noted above for Aspergillus fumigatus with respect to the effect of an increasing number of chloro substituents on the repression of fungal growth. An exception to this general trend was noted in the case of the veratrole derivatives (XXIV - XXVII, Table 2) where increasing substitution of chlorine appeared to exert no noticeable effect on fungal growth.

The data in Table 3 once again underscore the strongly inhibitory effect of 2,4,6-trichlorophenol. The potency of the latter compound as a toxicant is well-documented and it is found on the EPA's list of hazardous chemicals. At lower levels of chlorine substitution, the effect of the phenols on fungal growth was similar, provided the comparison was made between compounds having the same number of chloro substituents.

Among the six fungi tested, Cladosporium appears to be the least tolerant to the applied phenols. In general, however, this fungus and others are more resistant to phenols of the type tested than are fish, invertebrates, and other aquatic organisms.

Effect on the Alga Chlorella pyrenoidosa

The toxicity range of selected phenols toward an alga, Chlorella pyrenoidosa, was determined and the results obtained are presented in Table 4.

TABLE 4. TOXICITY RANGES OF SELECTED PHENOLS FOR CHLORELLA PYRENOIDOSA

Phenol	Concentration, ppm	
	Growth Present[a]	Growth Absent
Catechol (1,2-dihydroxybenzene)	20	40
2,4-Dichlorophenol (IV)	8	10
4,5-Dichlorocatechol (XXI)	2	4
4,5-Dichloroguaiacol (XIV)	1	10
Trichlorocatechol (XXII)	0.1	1

[a] Determined with a Spectronic-20 spectrophotometer and checked by cell count using a hemocytometer.

As in the previous tests with fungi, the enhancement of toxicity resulting from the introduction of chloro substituents on the phenolic ring is clearly evident. On the basis of the limited data in Table 4, the phenol concentration levels at which the Chlorella ceased to grow are considerably lower than those for similar compounds tested against fungi. For example, a concentration of 1 ppm trichlorocatechol completely inhibited the growth of Chlorella whereas in the case of the six fungi tested (cf. Table

3), the corresponding range was 7.5-50 ppm. Because of its greater sensitivity, Chlorella is therefore more suitable for use as a bioassay microorganism in the testing of low concentrations of phenols.

Effect on the Vascular Plant *Lemna perpusilla* (Duckweed)

Duckweed is a small aquatic plant that is widely distributed throughout the United States. Its utility as a test organism in acute toxicity bioassays was examined by determining the effect of various concentrations of four phenols on its growth. The results of these tests are shown in Table 5.

2,4,6-Trichlophenol (VI) was again found to cause the greatest suppression of growth in the limited series of phenols tested and, at a concen[illegible]ppm, it totally inhibited growth. The suppression of growth thereafter decreased in the order of 4,5-dichlorocatechol (XXI), 4,5-dichloroguaiacol (XIV), and 2,4-dichlorophenol (IV).

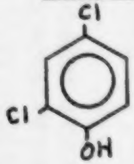
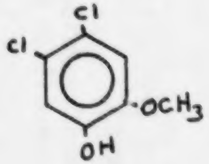
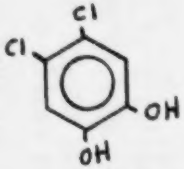
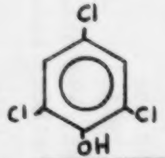
Based on a comparison of a limited number of phenols, therefore, *Lemna* appears to be more sensitive to such compounds than fungi, but less sensitive than *Chlorella*. However, duckweed is much easier to work with in the laboratory and as a biological test organism, it is less expensive to use.

Effect on *Daphnia magna*

The response of *Daphnia magna* to chlorophenols similar to or identical with those identified in spent chlorination and caustic extraction liquors is shown by the data compiled in Table 6. In a recent investigation, Durkin (24) has found that several of the phenols listed in this table interact with themselves, with certain unidentified components of spent chlorination liquor, and with sodium chloride in low concentrations, thereby altering somewhat the magnitude of the values reported in the table. This finding has important implications regarding the validity of attempts to account for the total toxicity of a particular liquor through the sum of effects of individual toxic components; nevertheless, the finding does not invalidate the more pronounced trends indicated by the data in Table 6.

One of the more consistent trends shown by the data in Table 6 is that the acute toxicity of the various chlorophenols increased in proportion to the number of chloro substituents on the phenolic ring. This same trend was also noted above in the discussion of the response of plant test organisms to many of the same phenols. As a class, and with respect to acute toxicity, the chlorocatechols (XVIII, XX-XXIII, Table 6) seem to have the greatest effect on the *Daphnia*. Somewhat surprisingly, 2,4,6-trichlorophenol (VI), which ranked as having a very toxic effect on the plant test organisms, proved to be somewhat less toxic than 3,4-dihydroxy-2,5,6-trichlorotoluene (XX) and 3,4,5-trichlorocatechols (XXII) in the tests with *Daphnia*. Chlorocatechols did, however, have a pronounced effect on retarding the growth of the plants exposed to them (see Tables 3-5).

TABLE 5. EFFECT OF FOUR PHENOLS ON THE VEGETATIVE GROWTH OF LEMNA PERPUSILLA[a]

Concentration, ppm		Number of Fronds, % of Control						
Phenol		0.0 (control)	0.1	1	5	10	15	20
	IV	100	-[b]	70	29	10	7	0[c]
	XIV	100	-	77	55	20	10	7
	XXI	100	95	95	20	0	-	-
	VI	100	94	31	0	0	-	-

[a] Ten days of incubation
[b] Not tested
[c] No growth

TABLE 6. ACUTE TOXICITY OF VARIOUS PHENOLS TO *DAPHNIA MAGNA*

Compound	24 hr LC_{50} or EC_{50} (95% confidence interval), μ moles/l	g[a]
2,4-Dichlorophenol IV	51.9 (40.2-90.1)[b]	0.424
2,4,6-Trichlorophenol VI	13.7 (11.4-16.0)[b]	0.206
4-Methylguaiacol VII	150 (76.1-1470)	0.403
6-Chloro-4-methylguaiacol VIII	49.9 (16.4-x[c])	0.905
5-Chloro-4-methylguaiacol IX	177 (19.5-x[c])	2.63
5,6-Dichloro-4-methylguaiacol X	13.0 (6.77-75.4)	0.477
2,5-Dichloro-4-methylguaiacol XI	25.5 (13.4-212)	0.438
2,6-Dichloro-4-methylguaiacol XII	14.6 (8.79-46.0)	0.290
2,5,6-Trichloro-4-methylguaiacol XIII	7.45 (2.74-x[c])	1.16
4,5-Dichloroguaiacol XIV	98.3 (79.0-135)[b]	0.309
4,5,6-Trichloroguaiacol XV	22.0 (15.6-26.5)[b]	0.261
Tetrachloroguaiacol XVI	4.96 (4.19-6.21)[b]	0.230
3,4-Dihydroxytoluene XVII	27.6 (21.0-36.5)	-[d]
3,4-Dihydroxy-6-chlorotoluene XVIII	13.8 (7.6-25.1)	-[d]
3,4-Dihydroxy-2,5,6-trichlorotoluene XX	4.90 (4.46-5.30)	-[d]
4,5-Dichlorocatechol XXI	6.64 (4.76-7.81)[b]	0.240
3,4,5-Trichlorocatechol XXII	3.39 (2.92-3.86)[b]	0.222
Tetrachlorocatechol XXIII	2.23 (1.25-2.80)[b]	0.319
4-Methylveratrole XXIV	1200 (557-2640)	0.472
5-Chloro-4-methylveratrole XXV	46.3 (20-x[c])	0.520
2,6-Dichloro-4-methylveratrole XXVI	20.7 (6.89-x[c])	0.873
2,5,6-Trichloro-4-methylveratrole XVII	2.36 (1.08-3.53)	0.262

[a] As discussed by Finney (11), the "g" value can be used as an index of the quality of the bioassay, i.e., how well the observed mortality pattern corresponds to the best estimate of the probit line. In general, "g" values of 0.4 or less indicate good agreement.

[b] LC_{50} values; all others, EC_{50}. The LC_{50} values are based on cardiac arrest whereas the EC_{50} values relate to immobility as response criteria.

[c] Meaningful upper limit not available due to low mortality at highest concentration tested.

[d] Not determined.

The isomeric dichloro-4-methylguaiacols (X-XII) show statistically insignificant differences in their LC_{50} values, a finding generally duplicated when fungi were used as test organisms (see Table 3). On the other hand, the effect of increasing numbers of chloro substituents that was apparent in increasing the toxicity of the veratroles (XXIV-XXVII) to Daphnia, was not observed in the treatment of the fungi with representatives of this class. Veratrole derivatives are atypical of the phenols detected in spent chlorination and caustic extraction liquors; the observed difference in the response of the fungi and Daphnia toward veratrole derivatives probably has little significance for our purposes in the present investigation.

ACUTE TOXICITY OF WHOLE AND FRACTIONATED SPENT CHLORINATION LIQUOR (SCL) AND CAUSTIC EXTRACTION LIQUOR (SCEL)

In this segment of the investigation, the toxicity of the whole spent chlorination and caustic extraction liquors was examined using a fungus (Aspergillus fumigatus) and an aquatic invertebrate (Daphnia magna) as test organisms. The validity of the use of fungi in acute toxicity bioassay has been critically discussed by Windaus and Petermann (25). Daphnia magna was selected on the basis of its comparative simplicity in bioassay applications and because it has been shown to give results that have a good correlation with bioassay data that is based on the utilization of fish (26).

In an effort to identify and define more thoroughly the chemical and physical characteristics responsible for toxicity, the whole liquors were subsequently fractionated by various means and the individual fractions subjected to acute toxicity bioassay and, to a lesser degree, chemical analysis. In this phase of the investigation, the contributions of the various fractions to the toxicity of the whole liquors were calculated using the "toxic unit" method described by Sprague (27). As defined by Sprague and applied throughout the course of this work, the toxic unit content was calculated as follows:

$$\text{Toxic Units (T.U.)} = \frac{\text{Total Organic Carbon (TOC) of Test Sample (ppm)}}{LC_{50}\text{ expressed as TOC (ppm)}}$$

Stability of Spent Chlorination Liquor (SCL) and Caustic Extraction Liquor (SCEL)

Before subjecting the spent chlorination and caustic extraction liquors to various fractionations, the stability of those liquors with respect to color, BOD_5, organically bound chlorine, and acute toxicity on storage was determined in order to evaluate the changes that would occur solely as a result of a particular form of treatment.

BOD_5 tests were performed on samples of the spent chlorination and caustic extraction liquors that ranged in age from 1 to 26 days. No significant trends were observed in either case, and the average values for spent chlorination and caustic extraction liquors were 125 and 437 mg O_2/l, respectively. As shown by the plots in Figure 3, the color of both caustic extraction liquors decreased at a greater rate initially than it did after prolonged standing. The difference in the color of the two extraction liquors

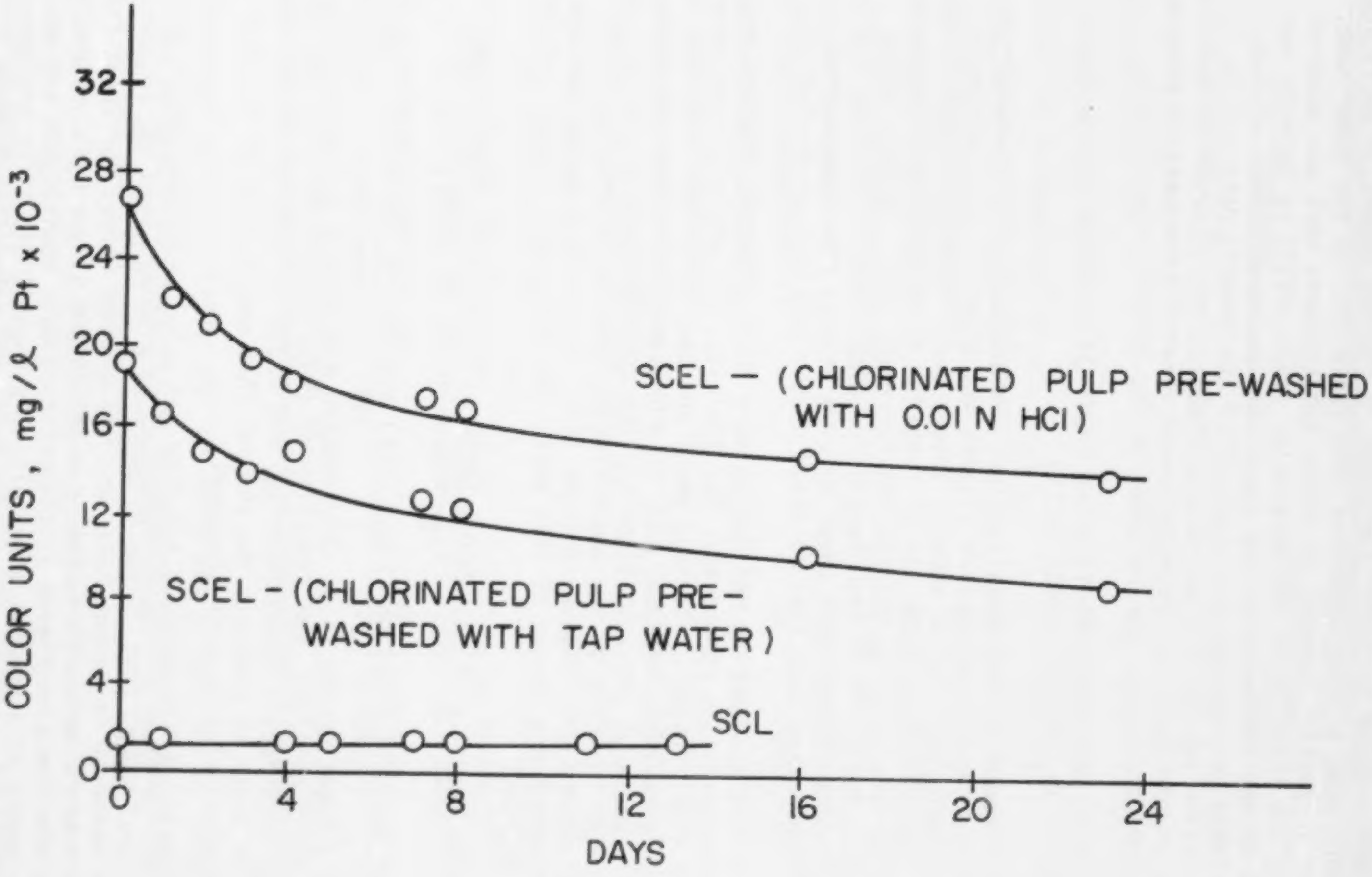


Figure 3. Effect of age on the color of spent chlorination and caustic extraction liquors stored at room temperature.

is attributed to the greater retention of solubilized material in the chlorinated pulp washed with dilute acid as compared to the one washed with tap water. In contrast, the color of the spent chlorination liquor remained virtually constant throughout the test period.

The stability of the organically bound chlorine in the two liquors is shown by the plots in Figure 4. The initial loss of chlorine in the case of the spent caustic extraction liquor is seen to be substantial at both 4° and 20°C. The spent chlorination liquor also lost organically bound chlorine on storage at room temperature but at approximately only 1/3 the rate observed for the caustic extraction liquor. The loss of organically bound chlorine was reduced as the pH of the liquor was progressively decreased.

In the acute toxicity determinations, samples of spent chlorination liquor (pH 2) were stored in the dark at 5°C and periodically tested against Daphnia magna. For periods of storage up to 39 days, the variations in 24-hr 24 LC_{50} values were not statistically significant. When allowed to stand at room temperature for 37 days, the same liquor likewise showed little significant change in acute toxicity. After 80 days' storage, however, a slight decrease in acute toxicity was observed, but further testing after more prolonged standing is required to confirm this trend. It is interesting to note, however, that should the trend toward decreased toxicity on standing be confirmed, it would parallel the decrease in organically bound chlorine content for the liquor noted above and might indicate a cause and effect relationship.

Spent caustic extraction liquor was also stored in the dark at room temperature and intermittently monitored for acute toxicity. After 80 days, no significant trend with respect to toxicity change was observed. In this situation, loss of organically bound chlorine appears to have had no effect on acute toxicity.

The apparent stability of the spent chlorination liquor with respect to acute toxicity is probably due, to a large extent, to its high acidity. As will be shown later, alkali treatment of this liquor had the effect of greatly decreasing its toxicity in a relatively short period of time. Smaller increases in the pH of spent chlorination liquor of the order of magnitude produced by diluting it with chlorinated pulp washings would undoubtedly have produced a similar effect, albeit over a greater period of time.

Collectively, the above findings indicate that although some of the properties of spent chlorination and caustic extraction liquors change on standing, acute toxicity testing can be performed on reasonably aged samples of such liquors without changes occurring in the course of the test.

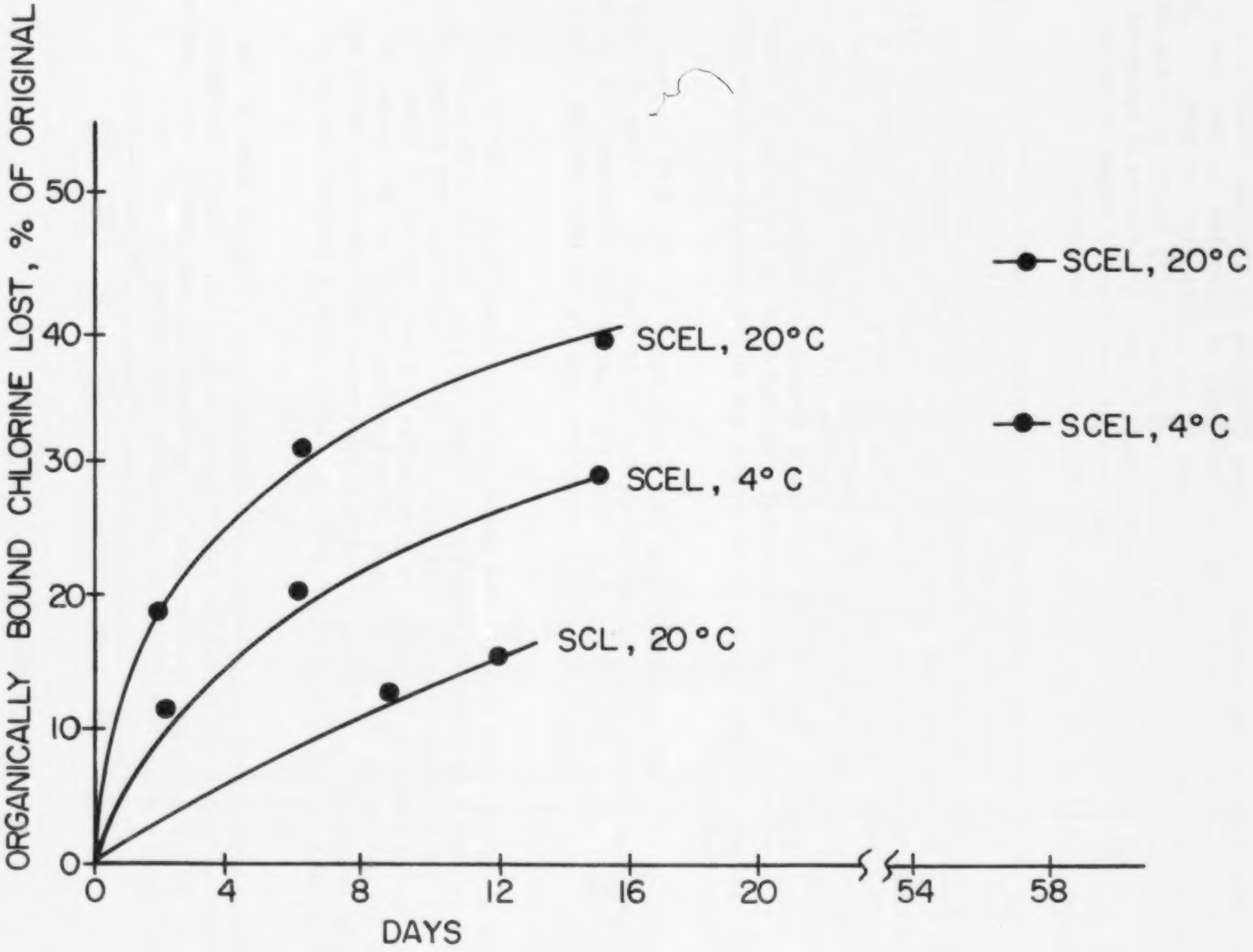


Figure 4. The rate of loss of organically bound chlorine for spent chlorination and caustic extraction liquors.

Responses of the Test Organisms to the Whole Spent Chlorination Liquor (SCL) and Spent Caustic Extraction Liquor (SCEL)

At their original concentrations, both the spent chlorination liquor and spent caustic extraction liquors were distinctly toxic to *Daphnia magna*. As seen in Table 7, the 24-hr LC_{50} value of the SCL is about six times smaller than that of the SCEL, which means that the SCEL is considerably less toxic than the SCL when compared on the same (TOC) basis. A similar result has been recently reported by Pfister and Sjostrom (28). The toxic units (TOC/LC_{50}) were calculated as 3.21 and 4.12 for SCL and SCEL, respectively. The total toxicity loads were calculated by multiplying the toxic units by the total liquor volumes which are regulated by the pulp consistencies of 3% and 10% for chlorination and caustic extraction, respectively. The toxicity loads are about 104 (SCL) and 37 (SCEL) toxic units per kg of pulp.

Table 7. ACUTE TOXICITY OF THE WHOLE SCL AND SCEL TO *DAPHNIA MAGNA*

	SCL[a]		SCEL[b]	
Test No.	TOC, ppm	24-hr LC_{50}, ppm of TOC	TOC, ppm	24-hr LC_{50} ppm of TOC
1	222	49	1492	419
2	249	67	1900	301
3	215	94	1577	472
4	249	96	1553	420
5	248	44	1621	440
6	230	43	1845	424
7	223	91	1624	412
8	214	90	1480	285
$\bar{X}$[c]	231	72	1637	397
σ[d]	15	24	155	67
s[e]	6	33	9	17

[a] spent chlorination liquor
[b] spent caustic extraction liquor
[c] $\bar{X}$ = mean value
[d] σ = standard deviation
[e] s = percent scattering

The pulp used in the present investigation was southern pine kraft. In many papers it is reported that the wood extractives, especially resin acids, play an important role in the toxicity of the spent pulping liquors to fish (21,29). To evaluate the possible contribution of the wood extractives to the SC and SCE liquors, the kraft pulp was thoroughly washed with water and then extracted successively with several portions of aqueous sodium hydroxide with intermediate washes under both ambient and elevated temperature conditions. Without making any attempt to identify and quantify all of the resin and fatty acids in the alkaline extracts, dehydroabietic acid was identified as its methyl ester in about 0.01% yield in the primary extract. The yields of total extracted material and methyl dehydroabietate decreased progressively with each extraction treatment. The complete removal of resin and fatty acids (extractives) from brown stock would thus appear to be unattainable under ordinary technical washing conditions.

To estimate the effect of extractives carried over from the brown stock into the spent chlorination and caustic extraction liquors on toxicity, the pulp was first extracted with ethanol-benzene solution (1:2) and then bleached under the same conditions as described in the Materials and Methods section. The resulting spent chlorination liquor showed a toxicity (24-hr LC_{50}, 60 ppm of TOC) which was comparable to the value found for SCL corresponding to the unextracted kraft pulp (see Table 7). The SCEL corresponding to the pre-extracted pulp showed, on the other hand, a slightly reduced toxicity (24-hr LC_{50}, 600 ppm of TOC) as compared to that found for the liquor corresponding to the unextracted pulp. Although the toxicity reduction was about 30% when calculated as toxicity units, the contribution to the total toxicity load (SCL + SCEL) was calculated to be less than 10%, thereby supporting the conclusion that the wood extractives and their chlorinated derivatives are minor contributors to the combined toxicity of SCL and SCEL.

The response of A. fumigatus to the original spent liquors is shown by the plots in Figure 5. The dry weight of fungal mycelium increased in the case of spent chlorination liquor. Because of the low concentration of solids in the original SCL, a sample of the same liquor concentrated to one-fifth of its original volume was also tested and a clear indication of growth stimulation was observed (Figure 5). On the other hand, the SCEL showed only a slight growth inhibition. Superficially, the results obtained with A. fumigatus seem to contradict those garnered by the use of Daphnia magna; i.e., neither of the two spent liquors appears harmful to the fungus, which seems to support the view which claims the invalidity of fungi as test organisms (25). However, as will be demonstrated below, this difference is more apparent than real and is a reflection of sensitivity differences in the test organisms.

Fractionation of the Spent Bleaching Liquors According to Molecular Size

Of the various separation methods available, those based mainly on molecular size differences, i.e., dialysis and gel permeation chromatography, were examined in order to determine the molecular size distribution of the toxicants in the spent bleaching liquors. Although it is essentially impossible to demonstrate a relationship between biological activity and individual chemical components without isolation and purification, attempts were made in the present investigation to effect a gross molecular size distribution of the active substances in spent bleaching liquor through a fractionation approach.

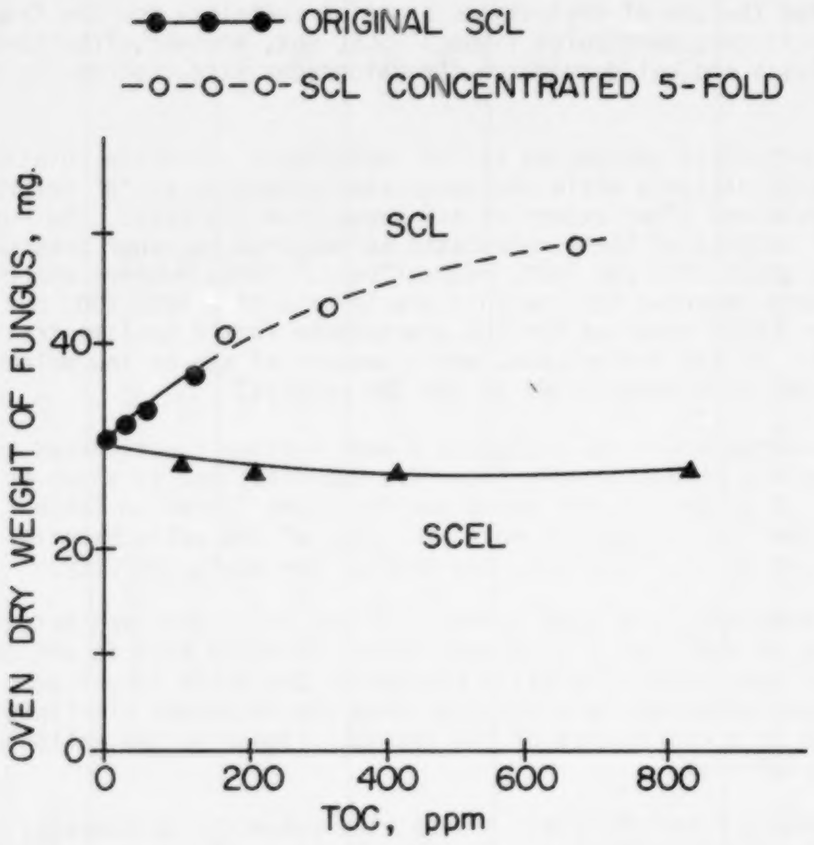


Figure 5. The response of Aspergillus fumigatus to the whole SCL and SCEL.

Dialysis of SCL gave two fractions. The maximum amount of material in the SCL passing through the cellophane tube (dialyzate) in a 24-hr period corresponded to 95% of the whole liquor. The corresponding value for the SCEL was 30%. This large difference may be taken as an indication that the SCEL contained a greater percentage of higher molecular weight material than the SCL. The large difference in the distribution of molecular sizes between the SCL and the SCEL was also evident from the patterns of their gel permeation chromatograms.

Failure of the cellophane membrane to retain more than 5% of the TOC in SCL negated the use of dialysis as a useful technique for the fractionation of solids in this particular liquor. SCEL was, however, fractionated using both dialysis and gel permeation chromatography with the results shown in Table 8.

The retentate designated as "A" indicates a retentate obtained after a single 24-hr dialysis while the retentate designated as "B" refers to a retentate obtained after extensive and repetitive dialysis. The number average molecular weights of these retentates as measured by vapor pressure osmometry were about 3100 and 7000, respectively. These numbers are close to the value (4000) reported for the acid precipitate of a SCEL (30) but larger than the number (500) reported for the precipitate formed by lime treatment of SCEL (31). In the latter case, small amounts of ash or low molecular weight organics may have contributed to the low results.

Both retentate A and dialyzate A were further fractionated by means of gel permeation chromatography into five sub-fractions as shown in Figure 6. The order of acute toxicity among the fractions listed in Table 8 indicates that the smaller the average molecular size of the acute material in the whole liquor or its fractions, the greater the acute toxicity.

The combined toxic unit contents of the dialyzates and retentates corresponding to the A and B treatment series revealed that 40 and 59%, respectively, of the number originally present in the whole liquor were unaccounted for. These losses may have resulted from the increased alkalinity in the dialysates as a consequence of the residual liquor in the cellophane tubes being concentrated.

Retentate A and dialyzate A were also subjected to bioassay using the fungus Aspergillus fumigatus. As shown by Figure 7, the oven dry weight of this fungus decreased when incubated with the concentrated dialysate. The large amount of scatter in the data may be taken as an indication that separation of materials into the dialyzate and retentate fractions was incomplete. The gel permeation chromatograms in Figure 6 appear to support this contention. Regardless, the sense of the data in Figure 7 is that certain growth inhibitors are present in the dialyzate of the SCEL; the effect of which becomes more pronounced as the concentration of materials in the test solution is progressively increased.

TABLE 8. ACUTE TOXICITY OF SCEL FRACTIONS TO *DAPHNIA MAGNA*

Separation Method	Liquor	Fraction	T O C		LC_{50} as TOC, ppm	Toxicity Units	
			ppm	% of Original		T.U.	% of Original
	Whole SCEL	-	1637	100	397	4.12	100
Dialysis	SCEL	Retentate A[a]	1065	65	1395	0.76	18
		Dialyzate A[a]	572	35	330	1.73	42
		Retentate B[a]	525	32	5000	0.11	11
		Dialyzate B[a]	1112	68	890	1.25	30
Gel Permeation Chromatography	Retentate A	R_1[b]	862	53	>1593	<0.54	<13
		R_2[b]	203	12	> 496	<0.41	<10
	Dialyzate A	D_1[b]	108	7	ND[c]	--	--
		D_2[b]	241	15	781	0.31	8
		D_3[b]	223	13	235	0.95	23

[a] A and B refer to single and multiple dialysis treatments, respectively.

[b] See Figure 6.

[c] Not determined

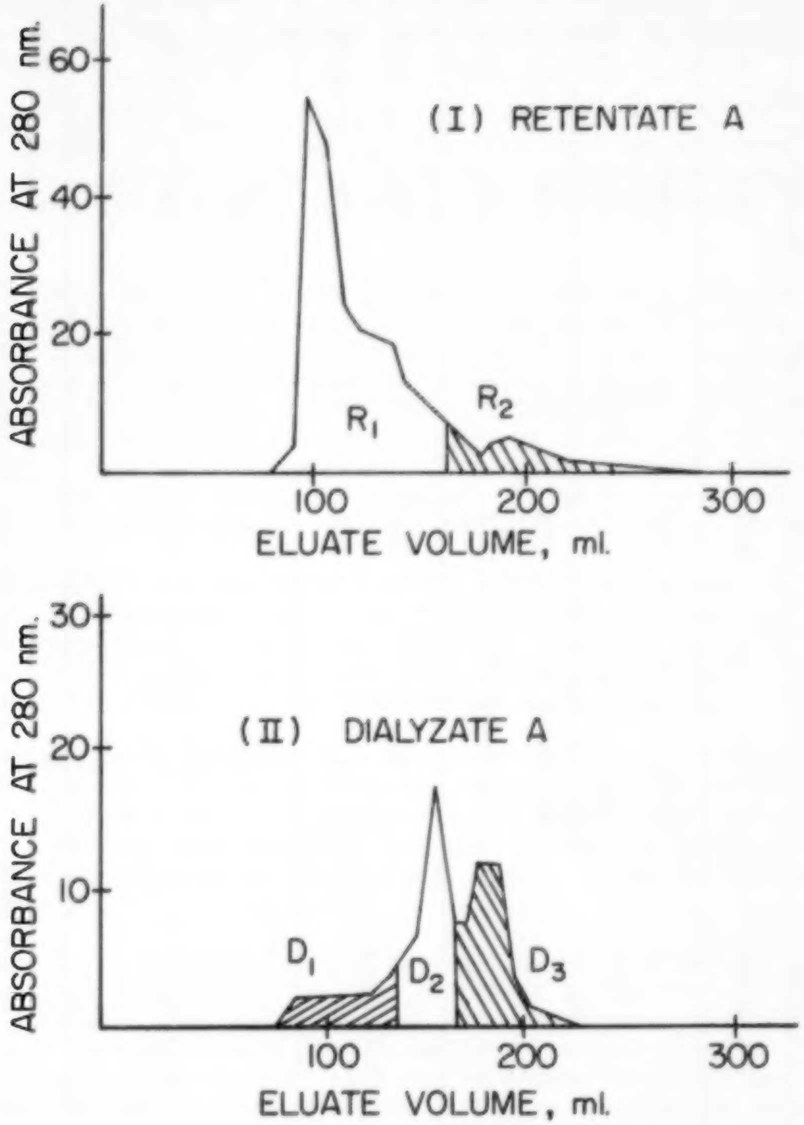


Figure 6. Gel permeation chromatogramsof retentate (I) and dialysate (II) with Sephadex G-25.

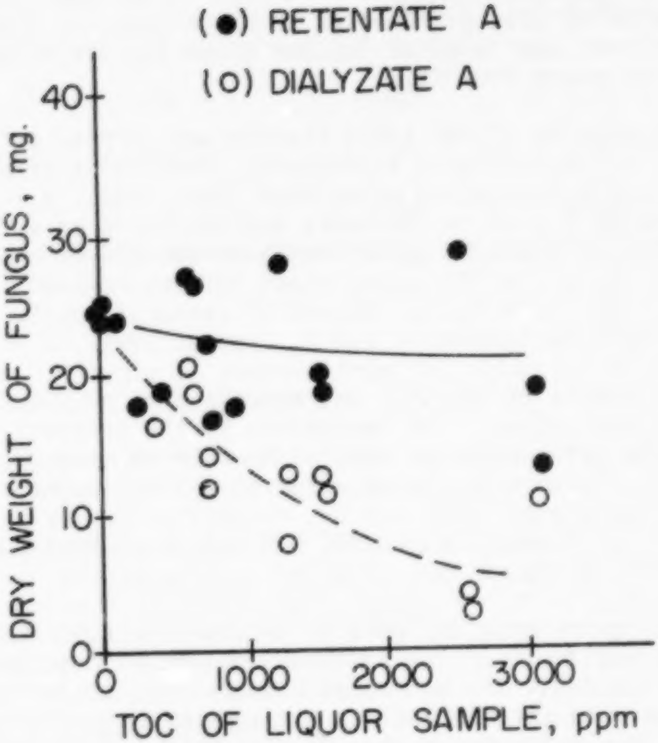


Figure 7. Relationship between the dry weight of fungus and the TOC of the fractions obtained by single dialysis.

Toxicity Characteristics of SCL and SCEL Ether Extracts

Solvent extraction and adsorption on activated carbon have been among the most frequently adopted techniques for the extraction of organic matter from water. Since desorption of materials adsorbed on activated carbon is sometimes difficult to achieve, the solvent extraction approach seemed preferable as a means of recovering the toxic materials from the spent liquors. Moreover, the results presented in the above section suggested that the toxic substances present in both spent chlorination and caustic extraction liquors were mainly low molecular weight compounds. In such a case, conventional solvent extraction appeared to be a logical approach for removing the toxic compounds from the spent liquors. Although it has been shown that ethyl acetate is superior to diethyl ether as an extractant for SCL and SCEL (32,33), the latter solvent was selected for use since the dissolved solids can be more safely recovered from it.

Ether extraction of the spent liquors was carried out for comparatively long periods in liquid-liquid extractors. The yields of extractives increased progressively with increasing extraction time. Thus, a 5-liter sample of the SCL was extracted for up to 196 hours and the yield of extractives at the end of this period was found to be 37% based on the TOC of the original liquor. Of the TOC extracted in 196 hours, about 80% was removed in the first 96 hours. Fractions collected at the end of consecutive 96- and 100-hr extraction periods were designated A_1 and A_2, respectively.

A 750-ml sample of the SCEL was acidified to pH 2 and similarly extracted for 184 hours with ether. The percentage of the original TOC recovered at the end of this extraction was about 20%. For the purpose of fractionation, the ether extracts were collected after 62 and 184 hours of consecutive extraction and designated as B_1 and B_2, respectively. As in the case of the SCL, the majority (about 70%) of TOC was extracted from the whole liquor within the first 62 hours.

As shown by the data in Table 9, most of the acute toxicity as shown by Daphnia magna was found in the initially collected fraction of each liquor (i.e., in A_1 and B_1). The extracted SCL was also subjected to acute toxicity bioassay after first removing trace residues of ether by means of a flash evaporator. The residual organics in extracted liquor represented 49% of the original TOC and showed an LC_{50} of 465 ppm of TOC. These results indicate that most of the substances toxic to Daphnia magna are extractable with ether. The LC_{50} value of 71 ppm of TOC for fraction B_1 (Table 9) indicates that ether extraction is more effective than the methods adopted previously (i.e., dialysis and gel permeation chromatography) for concentrating the toxic materials of the SCEL.

The response of Aspergillus fumigatus to the ether extracts of the SCL (A) and SCEL (B) and to the ether-extracted SCL (C) is shown by the plots in Figure 8. The yields of ether extracts for SCL and SCEL were, in this instance, 31% and 25%, respectively. As is clearly demonstrated by plots A and B, both ether extracts showed growth stimulation characteristics at lower concentrations of the extracts but distinct growth inhibition at higher concentrations.

TABLE 9. ACUTE TOXIC EFFECTS ON *DAPHNIA MAGNA* OF ETHER EXTRACTIVES OF SCL AND SCEL

Fraction	T O C		24-hr LC_{50}	Toxic units	
	ppm	% of original	ppm of TOC	T.U.	% of original
SCL[a]	231	100	72	3.21	100
A_1[b]	68	30	23	2.96	92
A_2[b]	15	7	28	0.54	17
SCEL[a]	1637	100	397	4.12	100
B_1[a]	218	14	71	3.07	75
B_2[c]	92	6	204	0.45	11

[a] Whole liquor (see Table 7)

[b] A_1 and A_2 correspond to material removed from SCL after consecutive 96- and 100-hr extraction periods, respectively.

[c] B_1 and B_2 correspond to material removed from acidified SCEL after consecutive 62- and 122-hr extraction periods, respectively.

Thus, there is an apparent parallel between the growth-inhibiting effect of the ether extracts on *Aspergillus fumigatus* and the acute toxicity displayed by the same extracts toward *Daphnia magna*, which is supportive of the conclusion that the toxic components of SCL and SCEL are ether extractable. The mutagenic compounds in SCL have likewise been found to be ether extractable (34). The finding that the ether extracts of SCL and SCEL display acute toxicity toward *Daphnia magna* at a concentration level where growth of *Aspergillus fumigatus* is stimulated probably is ascribable to a difference in the sensitivity of the two organisms. The fact that the ether-extracted SCL supported fungal growth may have been the direct result of the selective removal of a large percentage of the toxicants by ether extraction as already demonstrated.

Toxicity Characteristics of SCL Ether Extract Sub-fractionated on the Basis of Acidity

An ether solution of the extract from a freshly prepared sample of SCL (yield, 31%) was further separated into 1% $NaHCO_3$ soluble, 1% NaOH soluble, and neutral fractions (designated as A_a, A_b, and A_c, respectively). The response of *Daphnia magna* to these fractions are recorded in Table 10.

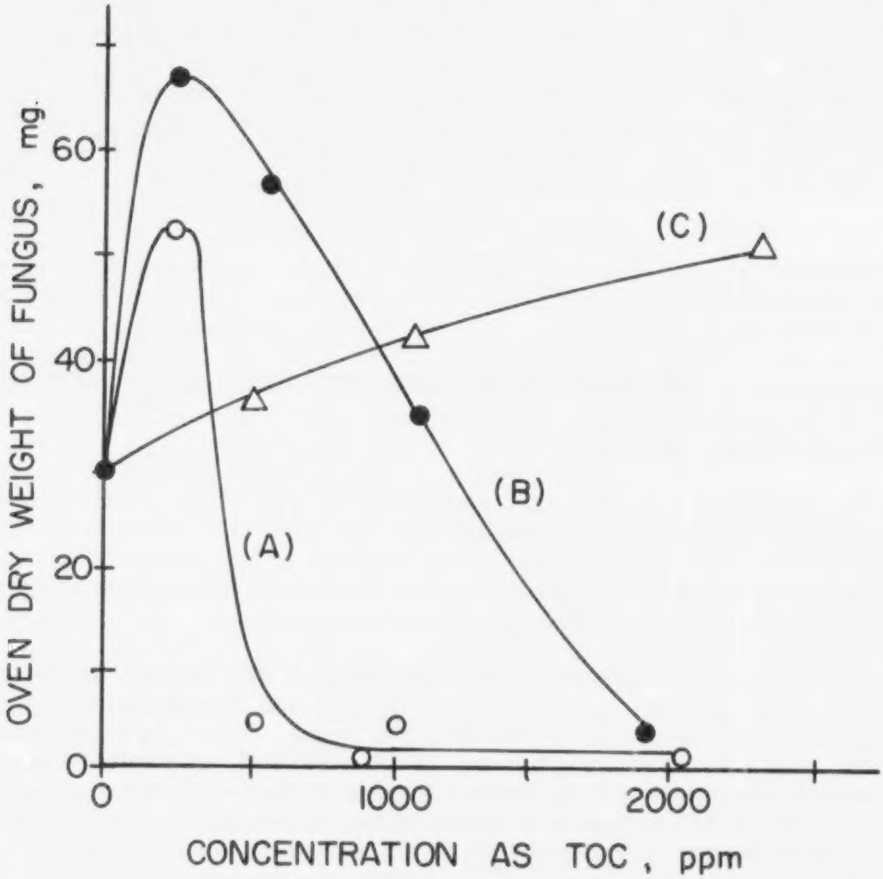


Figure 8. The effect of the ether extracts of SCL(A), SCEL(B), and of ether-extracted SCL(C) on the growth of *Aspergillus fumigatus*.

TABLE 10. TOXICITY OF THE SUB-FRACTIONS OF THE SCL ETHER EXTRACT AS INDICATED BY DAPHNIA MAGNA AND ASPERGILLUS FUMIGATUS

Symbol	Fraction	TOC[a]		24-hr LC_{50} ppm of TOC	Toxic units[a]		Cl w/w, %	Dry weight[b] of A. fumigatus in mg
		ppm	% of original		T.U.	% of original		
A	whole ether extract	72	31	29	2.48	77	25.0	1.5
A_a	weak acids	60	26	1500	0.04	1	16.0	43.5
A_b	very weak acids	7	3	23	0.30	9	6.8	39.7
A_c	neutrals	3	1	18	0.17	5	6.6	50.7

[a] Based on data for the whole SCL listed in Table 9

[b] Compared at a TOC concentration of 2800 ppm

As is apparent from the data in this table, most of the material in the whole ether extract was collected in the A_a (weak acid) sub-fraction. The compounds comprising this group are, in all likelihood, largely carboxylic acids. The acute toxicity of this sub-fraction is considerably lower than those of the whole ether extract and the very weakly acidic and neutral fractions. Although small in quantity, these latter fractions are highly toxic.

The compounds in sub-fraction A_b are probably phenolic in nature and several monohydric chlorophenols, chlorocatechols, and chloroguaiacols have, in fact, been identified by Lindström and Nordin (23) in SCL. Several of these compounds have been found to be acutely toxic to fish (21). Lindström and Nordin (35) have also identified a substantial number of neutral compounds (several sulfur- and chloro-containing), the toxicities of which have not been established in all cases. Presumably, sub-fraction A_c consists of compounds of the type identified by the above Swedish researchers.

The toxic unit contents of the three sub-fractions are substantially lower compared to that of the whole ether extract (Table 10). The loss in toxic unit content can be ascribed to reactions (other than neutralization) of certain components of the whole ether extract with the alkaline solutions ($NaOH$ and $NaHCO_3$) used in the sub-fractionation treatments. Similar reductions in acute toxicity have been observed when whole SCL has been made alkaline and subjected to bioassay using fish (36) and Daphnia magna (see later sections) as the test organisms. The data in Table 10 which indicate the three sub-fractions as being better able to support the growth of Aspergillus fumigatus than the whole extract support these findings. The discovery that the mutagenic properties of SCL are greatly reduced by alkaline treatment (34) is also important in this connection.

Although toxicity is often directly related to the chlorine content of a material, the data in Table 10 indicate that the sub-fraction with the highest chlorine content (A_a) also shows the lowest acute toxicity. In this instance, the type of structure(s) to which the chlorine is bound may have a greater influence on toxicity than chlorine content.

Toxicity and Chemical Characteristics of SCL and SCEL Ether Extracts Sub-fractionated by Adsorption Chromatography

The ether extracts designated A_1, A_2, B_1, and B_2 described in Table 10 were sub-fractionated by means of adsorption chromatography on silica gel. A typical elution diagram is shown in Figure 9. The response of Daphnia magna to the various fractions obtained by this type of separation is recorded in Table 11. The results in this table duplicate the finding illustrated previously by the data reported in Table 11, namely, that the toxic substances in SCL and SCEL are preferentially extracted by ether in the initial stages of the treatment. As is also apparent from the data in Table 11, most of the toxicity in A_1 and B_1 was recovered when these fractions were chromatographed on a silica gel column using ether as the elutant. Because these sub-fractions (A_1E and B_1E) represent a major fraction of the toxicity found in the whole SCL and SCEL, respectively, they appear to be excellent starting materials for further toxicity characterization.

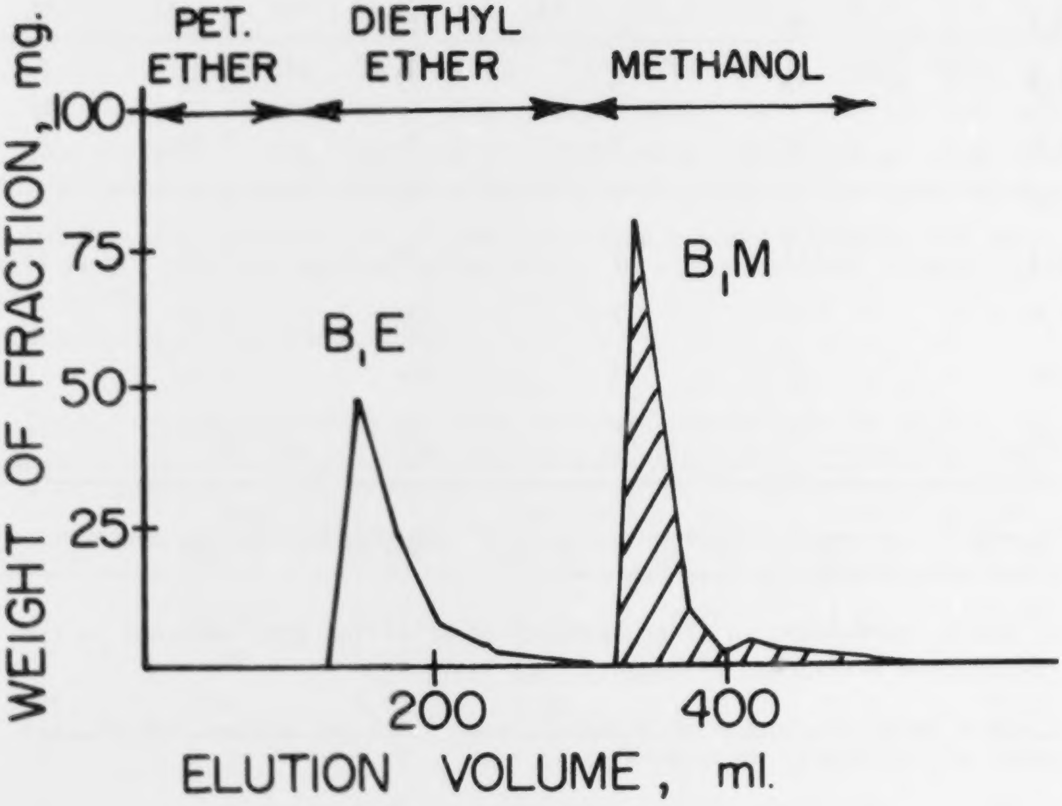


Figure 9. A typical elution diagram for the fractionation of SCEL ether extract on silica gel.

TABLE 11. TOXICITY CHARACTERISTICS OF SCL AND SCEL ETHER EXTRACTS FRACTIONATED BY CHROMATOGRAPHY ON SILICA GEL

Fraction[a]	T O C		24-hr LC_{50} ppm of TOC	Toxic units	
	ppm	% of original		T.U.	% of original
SCL[b]	231	100	72	3.21	100
SCEL[b]	1637	100	397	4.12	100
A_1E	50	22	15	3.33	104
A_1M	18	8	77	0.23	7
A_2E	10	4	31	0.32	10
A_2M	4	2	38	0.11	3
B_1E	107	7	39	2.74	67
B_1M	111	7	138	0.80	20
B_2E	61	4	164	0.37	9
B_2M	31	2	47	0.66	16

[a] A_1 and A_2 correspond to ether extracts of SCL obtained in two consecutive extraction treatments (see Table 9).

B_1 and B_2 correspond to ether extracts of acidified SCEL obtained in two consecutive extraction treatments (see Table 9).

E and M refer to elution of material from silica gel column with diethyl ether and methanol, respectively.

[b] Data correspond to the mean values shown in Table 7.

In comparison with the sub-fractions of SCL ether extractives eluted from a silica gel column with methanol (A_1M), the corresponding sub-fraction from SCEL (B_1M) displayed a high toxic unit content. As suggested by results discussed in a previous section, the difference may be the result of a greater contribution to total toxicity (in the case of SCEL) by wood extractives.

Elemental and functional group analyses were performed on the ether extracts of SCL and SCEL (∿260 hours of extraction) and on sub-fractions obtained by chromatography on silica gel with the results shown in Tables 12 and 13. The only significant differences among the fractions are found in

TABLE 12. ELEMENTAL COMPOSITION OF THE SCL(A) AND SCEL(B) ETHER EXTRACTS AND ETHER EXTRACT SUB-FRACTIONS

Fraction[a]	Yield,[b] %	$\overline{M}n$	Weight, % C	Weight, % H	Weight, % Cl	Weight, % O	Number/$\overline{M}n$ C	Number/$\overline{M}n$ H	Number/$\overline{M}n$ Cl	Number/$\overline{M}n$ O
A	58.6	292	38.7	3.4	23.0	34.9	9.4	9.7	1.9	6.4
AE	27.5	307	39.1	3.6	23.2	34.1	10.0	11.0	2.0	6.6
AM	31.1	793	36.6	3.4	15.3	44.7	24.2	26.9	3.4	22.1
B	21.8	225	46.4	4.4	7.4	41.8	8.7	9.8	0.5	5.9
BE	6.7	256	50.1	5.0	6.1	38.8	10.7	12.7	0.4	6.2
BM	15.1	746	44.0	3.6	7.6	44.8	27.3	26.8	1.6	20.9

[a] A and B = ether extracts of SCL and SCEL, resp.; E and M = ether and methanol, resp., solvents applied consecutively to eluted material from silica gel column.

[b] Based on TOC of the whole liquors.

TABLE 13. ACIDIC GROUP AND TOTAL HYDROXYL CONTENTS OF THE SCL(A) AND SCEL(B) ETHER EXTRACTS AND ETHER EXTRACT SUB-FRACTIONS

Fraction[a]	Weak acids meq./g	Weak acids No./$\overline{M}n$	Very weak acids meq./g	Very weak acids No./$\overline{M}n$	Total hydroxyl meq./g	Total hydroxyl No./$\overline{M}n$
A	3.79	1.11	2.60	0.76	4.82	1.41
AE	4.07	1.25	2.04	0.63	5.00	1.54
AM	3.91	3.10	2.50	1.98	5.18	4.11
B	6.70	1.51	1.99	0.45	5.67	1.28
BE	7.58	1.94	2.00	0.51	6.27	1.61
BM	2.77	2.07	1.94	1.45	5.31	3.96

[a] Symbols identified in footnote a, Table 12.

the chlorine content and number average molecular weight values. The higher chlorine content of the SCL ether extract as compared to that of the SCEL ether extract may explain the greater toxicity of the former. Comparison of the number average molecular weight values listed in Table 12 for the E and M sub-fractions with the appropriate acute toxicity data in Table 11 shows that greater toxicity is associated with the sub-fractions composed of lower average molecular weight material as was speculatively assumed previously in this paper on the basis of less firm data.

The results presented in this section have provided evidence indicating that all or most of those compounds in SC and SCE liquors contributing to toxicity are of low molecular weight and as such may be conveniently separated from the non-toxic substances in these liquors using separation techniques based on molecular size. Of the fractionation approaches attempted, ether extraction proved to be one of the more satisfactory for effecting the non-destructive recovery of low molecular weight (i.e., toxic) substances in SCL and SCEL. This achievement is particularly important in the case of SCL which is especially prone to undergo changes which affect its toxicity. Ether extracts of SCL and SCEL therefore represent excellent starting materials for further work relating to toxicity.

DEGRADATION OF PHENOLS BY CHEMICAL TREATMENT

Treatment with Ozone

With respect to its reactions with organic materials, ozone is best known for its ability to react with and ultimately cleave aliphatic carbon-carbon double bonds (37,38). When applied to spent bleaching liquors, ozone could therefore be expected to react with fatty acids and lignin fragments containing such groups. Although less readily attacked than aliphatic carbon-carbon double bonds, phenols as a class of compounds are reactive toward ozone (39). Published findings (39-46) consistently indicate that ozone functions by breaking down the aromatic rings of lignin and simple phenols forming, ultimately, aliphatic carboxylic acid fragments.

An inspection of relevant literature reveals that of the phenols reacted with ozone, relatively few possessed aromatically bound chloro substituents and even these were atypical of chlorophenol types identified thus far in spent chlorination and caustic extraction liquors (21-23). In order to obtain information more relevant to the situation actually encountered in spent chlorination and caustic extraction liquors, chlorophenols representative of the phenolic types identified in such liquors were ozonized in a slightly acidic aqueous medium and their rates of disappearance individually determined.

Sufficient amounts of the aforementioned phenols were dissolved in ethanol/water solutions to provide 0.414 mM solutions. After the flow rate had been separately determined, the ozone was introduced into the solution of phenol through a fritted glass diffuser tube. After varying amounts of ozone had been applied, the residual phenol was determined by means of gas chromatography and ionization difference spectroscopy. A detailed description of the apparatus and procedures is provided in the EXPERIMENTAL section.

Calculation of Ozonization Rate Constants--

The procedure used to obtain rate constants for the reaction of ozone with the various phenols was the same as that developed by Gould and Webber (45). The system is assumed to be a simple one:

$$\text{Phenol} + O_3 \xrightarrow{ka} \text{Products} + O_2$$

The rate expression the authors derived for this process is as follows:

$$\ln (Ph_0/Ph) = ka\ Dt$$

where Ph_0 = initial concentration of phenol (moles/l)

Ph = concentration of remaining phenol (moles/l)

ka = rate constant (moles of phenol removed/mole of ozone)

D = dose rate (moles of ozone fed per minute/mole of phenol per liter initially present)

t = time (minutes)

The plot of the natural log of Ph_0/Ph versus Dt yields a straight line with a slope equal to ka. The rate constants were calculated by taking all the values of Ph_0/Ph and Dt for each sample withdrawn from the reaction vessel, up to the point where 60% of the starting compound had reacted. This value was chosen as the upper limit to avoid interference in the ultraviolet spectroscopic measurements of residual phenol by accumulating degradation products. The slope (ka) was then determined using an HP-55 calculator with automatic linear regression, in order to eliminate the errors inherent in a graphical method. Ideally, it would have been desirable to use only those points corresponding to the first 20% of phenol removal, but in many instances the initial reactions were so rapid that insufficient data were available to allow the slope to be determined with any degree of accuracy.

Effect of Structure on Ozonization Rate--

The rate constants for the reaction of ozone with various model phenols are compiled in Table 14. Initially, the residual phenol concentrations were measured gas chromatographically. This approach proved to be time consuming, however, because of the large number of samples involved and the time needed to prepare each sample for analysis. Ultraviolet spectroscopy based on measurement of ionization difference spectra appeared to represent a much simpler and more convenient alternative approach. Analyses subsequently performed on the ozonization product mixture of trichlorocreosol (XIII) indicated that the yields of residual phenol, as determined by the spectroscopic and chromatographic methods, agreed to within 2%. Accordingly, the UV spectroscopic method was thereafter adopted for use in determining the residual phenolic content of phenol/ozone product mixtures. The gas chromatographic method continued to be used for the determination of veratrole derivatives (XXIV-XXVII) since they have no ionizable groups.

TABLE 14. OZONIZATION RATE CONSTANTS OF PHENOLS

Compound[a]	Initial pH	Final pH	Moles of ka phenol removed/mole O_3[b]
I	6.15	6.05	0.90
II	6.15	5.85	0.83
V	6.15	5.83	2.44
VII	6.15	5.85	2.43
VIII	6.15	5.45	1.62
IX	6.15	5.90	1.11
X	6.15	5.42	2.02
XI	6.15	5.68	2.04
XII	6.15	5.30	2.56
XIII	6.15	5.20	2.61
XVI	10.3	> 10	∿13.6
XVII	6.15	5.65	3.72
XVIII	6.15	5.29	2.53
XIX	6.10	6.05	5.25
XX	6.15	6.10	4.42
XXIV	6.15	5.65	1.77
XXV	6.15	6.15	0.20
XXVI	6.14	6.14	0.02
XXVII	6.15	6.15	-[c]

[a] See Table 2 for structures.

[b] Compound XVI: initial conc., 1.01 mM; O_3 flow rate, 0.72 mg/min.
All others: initial conc. 0.414 mM; O_3 flow rate, 2.5 mg/min.

[c] No reaction after application of 27 mg of O_3.

As was stated previously, only data corresponding to the removal of up to 60% of the original phenol were used to calculate the rate of constants in order to reduce the possibility of phenol oxidation products interfering with the measurements. In a number of instances, differential absorbance was noted at wavelengths slightly higher than the absorption maxima found for the phenols (297-313 nm). This differential absorbance increased with increasing ozone input, thereby indicating the resistance of the contributing structure(s) to breakdown by the oxidant.

In general, the data in Table 14 indicate that the reactivity of the phenols toward ozone decreases in the order: catechols (XVII-XX), guaiacols (VII-XIII), cresols (II and V), and veratroles (XXIV-XXVII). This sequence is consistent with the order generally observed for the reactivity of oxidants, including a sparse amount of information relating specifically to ozone (47), toward mono- and dihydric phenols and their ethers.

The rates of reaction of phenols of the guaiacol (VII-XIII) and catechol (XVII-XX) types with ozone decrease with the introduction of one chloro substituent onto the ring. This finding is predictable since ozone functions as an electrophile (see ref. 48) and the chloro substituent reduces the electron density of the ring. However, as the number of chloro substituents increased, the rate constants (cf. Table 14) actually showed a progressive, albeit a somewhat erratic, increase rather than the expected decrease. An explanation for this apparent anomaly is not readily discerned from the data at hand. However, one working hypothesis can be evolved based on the statements of other investigators (39,48,49) indicating that the intermediate oxidation products of phenols with ozone (e.g., o-benzoquinones and muconic acids) are themselves readily attacked and degraded by ozone. Thus, there exists a competition for ozone between residual phenol and its breakdown products. In the case at hand, it is conceivable that the effect of chloro substituents on reducing the rate of ozone attack is greater when they are present on phenol breakdown products than when they are situated on phenolic rings. In applying this interpretation to the present situation, it has to be assumed that the number and possibly the position of the chloro substituents on both the phenol and its breakdown products are critical variables.

Comparison of the rate constants (Table 14) for the reaction of phenol itself (I) and p-cresol (II) reveals that the effect of H and CH_3 substituents at the position para to the phenolic hydroxyl is not pronouncedly different. Contrary to the behavior displayed by phenols VII-XIII and XVII-XX, introduction of one chloro substituent onto the cresol ring did not result in a reduction of the reaction rate with ozone, as was observed with phenols of the guaiacol and catechol types. The reaction rate did, however, increase systematically as the number of chloro substituents increased from 0 to 2. The reaction sequence proposed (42,45,48,50) for the oxidative breakdown of monohydric phenols with ozone involves a prior conversion to a catechol through hydroxylation at a position ortho to the phenolic hydroxyl group. Whether this additional step is sufficient to explain the slightly different effect of chloro substituents on the observed rates of mono- and dihydric phenol ozonization cannot be determined with the evidence at hand. The problem is further complicated by the fact that conversion of monohydric phenols to catechols may be prevented by the presence of chloro substituents at the positions ortho to the phenolic hydroxyl group.

The fully etherified phenols (XXIV-XXVII) are atypical of the phenols identified thus far in spent chlorination and caustic extraction liquors (21-23) but were included in the study for purposes of comparison with other phenolic types and with the hope of obtaining information regarding the mechanism of oxidative breakdown with ozone. The rate data in Table 14 clearly demonstrate the relative unreactivity of these compounds toward ozone.

The rate constants in this series of compounds decrease progressively with increasing substitution of chlorine as would be predicted assuming an electrophilic attack of ozone and no other complicating side effects.

The observed stability of the trichloroveratrole derivative (XXVII) toward ozone contrasts sharply with the behavior of the corresponding guaiacol and catechol compounds XIII and XX, respectively, both of which were extensively degraded by ozone under the same conditions. Other evidence indicating that a different mode of aromatic ring degradation is operative in the ozonization of veratrole derivatives lies in the observation that the ozonization product mixtures of such compounds were colorless whereas those of the guaiacols and catechols were initially highly colored. This finding suggests that aromatic rings in veratrole-type structures are not degraded through an o-benzoquinone intermediate.

In other studies (41,46) of the reactions of similar (veratryl-type) compounds with ozone, the product analysis showed that, similar to the behavior of the guaiacol and catechol types, ring scission occurred mainly between the oxygen-bearing carbons. It is important to note, however, that this is not exclusively the case since Kratzl et al. (46) have detected dimethyl oxalate among the ozone oxidation products of veratrole and 4-methylveratrole (XXIV). This finding indicates a rupture of the bonds between carbon atoms 2 and 3 and 4 and 5 and represents a complicating factor in attempting to rationalize the effect of structure on ozonization rate.

In contrast to the other compounds listed in Table 14, tetrachloroguaiacol (XVI) was ozonized in an alkaline (pH 10.3) medium and under slightly different conditions with respect to initial concentration and ozone flow rate than those employed with the former set. Although the ozone treatment of tetrachloroguaiacol was not performed under the rigorously controlled conditions required for the acquisition of reliable kinetic data, a rate constant for the reaction was nevertheless calculated (Table 14). After making ample allowance for the potential inaccuracies of the measurement, the value reported for the rate constant is still sufficiently higher than the rate constants reported for the other phenols to justify the conclusion that the reaction of tetrachloroguaiacol with ozone is enhanced in an alkaline medium. Support for this interpretation is well documented in the literature where several investigators (40,45,49,50) concluded that phenols are oxidized in preference to other substances in alkaline media. The effect of the alkali may be ascribed to an enhancement of the electron density on the ring as a consequence of forming a phenolate anion, thereby promoting the electrophilic attack of ozone. An alternative or complementary explanation is that, in aqueous, alkaline media, ozone is decomposed into hydroxyl radicals (51) which are more reactive toward phenols than ozone itself.

The relationship between the extent of removal of tetrachloroguaiacol and the acute toxicity of the product solutions toward *Daphnia magna* is shown by the data in Table 15.

TABLE 15. DETOXIFICATION OF TETRACHLOROGUAIACOL (XVI) BY OZONIZATION AT pH 10

Ozone consumed/ initial phenol onc.[a] (mmoles/mmole)	Phenol loss, %	24-hr LC_{50} ppm based on original phenol	24-hr LC_{50} ppm of carbon
0	0	0.22	0.06
1.57	50	4.1	1.31
5.13	100	>10	>3.21

[a] Initial conc. of phenol, 259 mg/l; ozone flow rate, 0.60 mg/min.

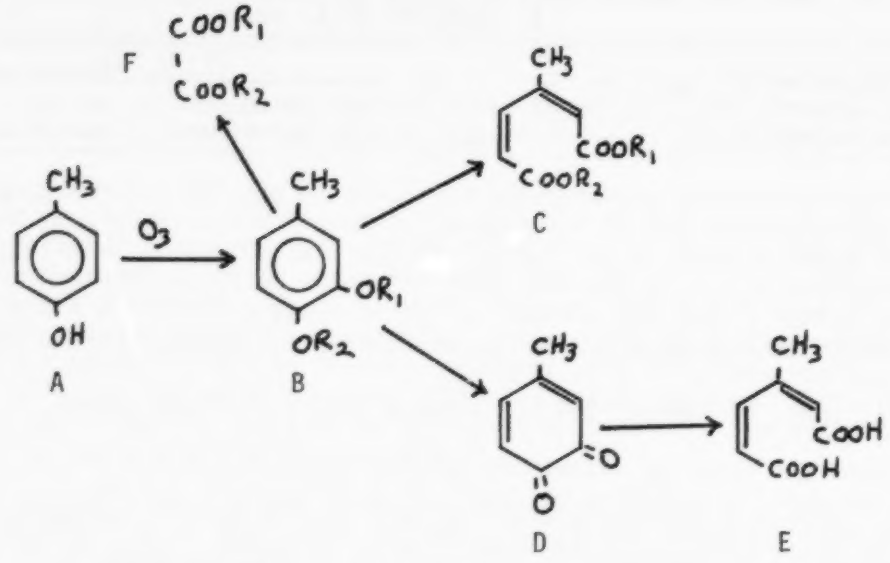
It is apparent from the values listed that tetrachloroguaiacol can be totally removed from solution by treatment with ozone in an alkaline medium. The decrease in phenol content was roughly paralleled by a decrease in acute toxicity as indicated by the response of Daphnia magna to the ozonized solutions. It is also important to note that when phenol removal was complete, the product mixtures were essentially non-toxic. A similar finding has been reported by Niegowski (40).

Extent of Phenol Removal--

Continued ozonization of the phenols and phenol ethers resulted in extensive breakdown of these compounds. Although ozonization was terminated short of 100% removal of the phenol, evidence was obtained suggesting that this goal was attainable in all but a few instances. The fully etherified models (XXIV-XXVII) proved considerably more resistant to oxidative breakdown than the other classes of phenols tested, and their stability increased with increasing substitution of chlorine on the ring. In the extreme case, 4-methyl-trichloroveratrole (XXVII) was recovered essentially intact after the application of a considerable excess of ozone.

The efficiency of phenol removal appeared to decrease with increasing application of ozone. This situation was seemingly the result of the formation of increasing quantities of phenol oxidation products which competed successfully with residual phenol for the ozone. Because of such complexities, few if any consistent trends were apparent in the residual phenol/ozone consumption data, and attempts to draw any reliable conclusions regarding the relationship between the ozone required to effect complete removal of the phenols and their structure were unsuccessful.

The primary intermediate breakdown products of phenol oxidation by ozone have not been extensively investigated. The sequence shown below is a composite which combines experimental findings with the speculations of a number of investigators.



Monohydric phenols (represented by A) are believed to first undergo hydroxylation to a catechol ($B;R_1=R_2=H$). The aromatic ring of B structures subsequently may undergo further attack by ozone directly forming a muconic acid derivative (C) through rupture between the oxygen-substituted carbon atoms. In the case of 4-methylveratrole ($B;R_1=R_2=CH_3$), Kratzl et al. (46) have actually identified dimethyl cis,cis-β-methylmuconate among the reaction products. Alternatively, cis,cis-β-methylmuconic acid (E) may be formed via an o-benzoquinone intermediate (D). Since compounds of the latter type are colored, the formation of colored ozonization reaction mixtures could be taken as evidence for the reality of this portion of the above sequence.

In the present investigation, ozonization of all compounds except V, XXIV, XXVI and XXVII resulted in the formation of colored product mixtures, thus supporting the concept that at least a portion of the overall degradation of the majority of phenols tested occurred via an o-benzoquinone intermediate. The color initially generated by the introduction of ozone into the phenol solutions was discharged when 0.8 to 2.0 moles of O_3/mole of phenol was consumed. One group of investigators (49) has suggested that oxygen as a decomposition product of ozone plays a role in the overall process of phenol degradation.

Another pathway in the degradation of phenols with ozone consists of the breaking of carbon-carbon bonds other than those between the oxygen-bearing carbons. Thus, Kratzl et al. (46) identified dimethyl oxalate ($F;R_1=R_2=CH_3$) in the product mixture from the ozonization of 4-methylveratrole (XXIV) in aqueous acetic acid. Bernatek et al. (47) have also postulated ozone attack on a phenol ring at sites other than the oxygen-substituted carbons.

The reactivity of o-benzoquinones, muconic acids, and other possible phenol breakdown products toward ozone in comparison with that of phenols toward the same oxidant has not been extensively investigated. Generally, application of the theoretically required amount of ozone to eliminate completely a given quantity of phenol has been found to be insufficient for this purpose (42,44,45,47). This finding has been interpreted as indicating a greater reactivity of ozone toward the primary ozonization products than toward the starting material (47).

A similar competition exists in the spent bleaching liquors where the simple phenols are mixed with phenolic breakdown products, larger lignin fragments, and carbohydrates. The specificity of ozone for a monomeric phenol was tested by diluting an aqueous solution of tetrachloroguaiacol (XVI, Table 2) containing 0.1 mmole of the latter with increasing amounts of spent caustic extraction liquor and reacting the mixtures with a fixed amount of ozone. The results of this experiment are compiled in Table 16.

TABLE 16. OZONE OXIDATION[a] OF TETRACHLOROGUAIACOL-SPENT CAUSTIC EXTRACTION LIQUOR COMBINATIONS

Carbon contributed by tetrachloroguaiacol, % of TOC	Tetrachloroguaiacol removed, %
100	81
30.7	69
12.8	44
4.2	14

[a] Ozone applied, 5.28 mg; ozone flow rate, 0.66 mg/min.; pH 10.3

These results reveal that the organic compounds present in the spent caustic extraction liquor do indeed compete effectively with the added tetrachloroguaiacol for ozone. The bottom values in each of the two columns probably come closest to approximating the situation existing in spent caustic extraction liquor with respect to the fraction of total organic carbon (TOC) contributed by the phenolic component. In this situation, the removal of tetrachloroguaiacol was far from quantitative. However, comparison of the corresponding values in the two columns reveals that the removal of tetrachloroguaiacol (14%) was nevertheless greater than would have been expected on the basis of its contribution to the total carbon of the system (4.2%) and some specificity of the ozone for the phenol is indicated. However, this preference is moderated and controlled by the relative concentration of the phenol and ozone, and the reactivity of the other liquor components toward ozone.

Another parameter reflecting the character and extent of the ozonization treatment is the formation of chloride through oxidative breakdown of phenols containing organically bound chlorine. In the present study, the concentrations of chloride ion in the ozonized solutions of the chlorophenols were determined as a function of the amount of applied ozone. The results of these analyses revealed that chloride ion was produced rapidly and extensively after the addition of only a small amount of ozone to phenols of the guaiacol, catechol, and cresol types. Low yields of chloride from the veratrole derivatives again indicated their poor reactivity toward ozone. In the extreme case, no chloride was detected when 3,4-dimethoxytrichlorotoluene (XXVII) was ozonized.

The percent recovery of chloride based on theoretical yield was greatest (over 60%) for the monochloro compounds VIII, IX, XVIII, and XXV. The dichloro and trichloro derivatives all produced chloride ion yields in the range 30 to 50% of theoretical; individual differences were too small to relate to structural features with any degree of confidence. In no case did the yield of chloride ion approach the theoretical value for 100% removal, and it must be presumed that chlorine-containing, ozone-resistant breakdown products were formed. This conclusion is supported by the results of a study by Gilbert (44),who ozonized several monohydric chlorophenols and recorded chloride yields ranging from 60-97% when approximately 4 moles of ozone/mole of phenol were applied.

Treatment with Chlorine Dioxide

The ability of chlorine dioxide to react with and degrade phenolic lignin model compounds is well documented in the literature (4, 52-55). This fact, coupled with the current industry trend toward greater utilization of chlorine dioxide in the first bleaching stage provided the impetus for performing a few experiments involving the reactions of chlorine dioxide on phenols similar to or identical with those detected in spent chlorination and caustic extraction liquors.

In these experiments, selected phenols (conc., 10 mM) were reacted with varying amounts of chlorine dioxide in the dark at ambient temperature until the oxidant was completely consumed. The residual phenol was recovered by extraction of the product mixture with chloroform and the amount determined by gas chromatography. The results are shown by the plots in Figure 10. The plots indicate the likelihood of completely removing the phenols tested, provided sufficient amounts of chlorine dioxide are applied. With the possible exception of 2,4-dichlorophenol (IV), the responses of the phenols show no pronounced differences. Since either 2 or 4 oxidizing equivalents are theoretically required to convert the various phenolic rings in question to non-aromatic moieties, it is apparent that the removal of the phenols tested is a very inefficient process.

The efficiency of phenolic ring breakdown by chlorine dioxide was further tested by applying a fixed amount of chlorine dioxide to a solution of tetrachloroguaiacol (XVI) diluted with increasing amounts of spent caustic extraction liquor. The results (see Table 17) reveal a decrease in the detoxification factors ranging from 0.701 to 0.826.

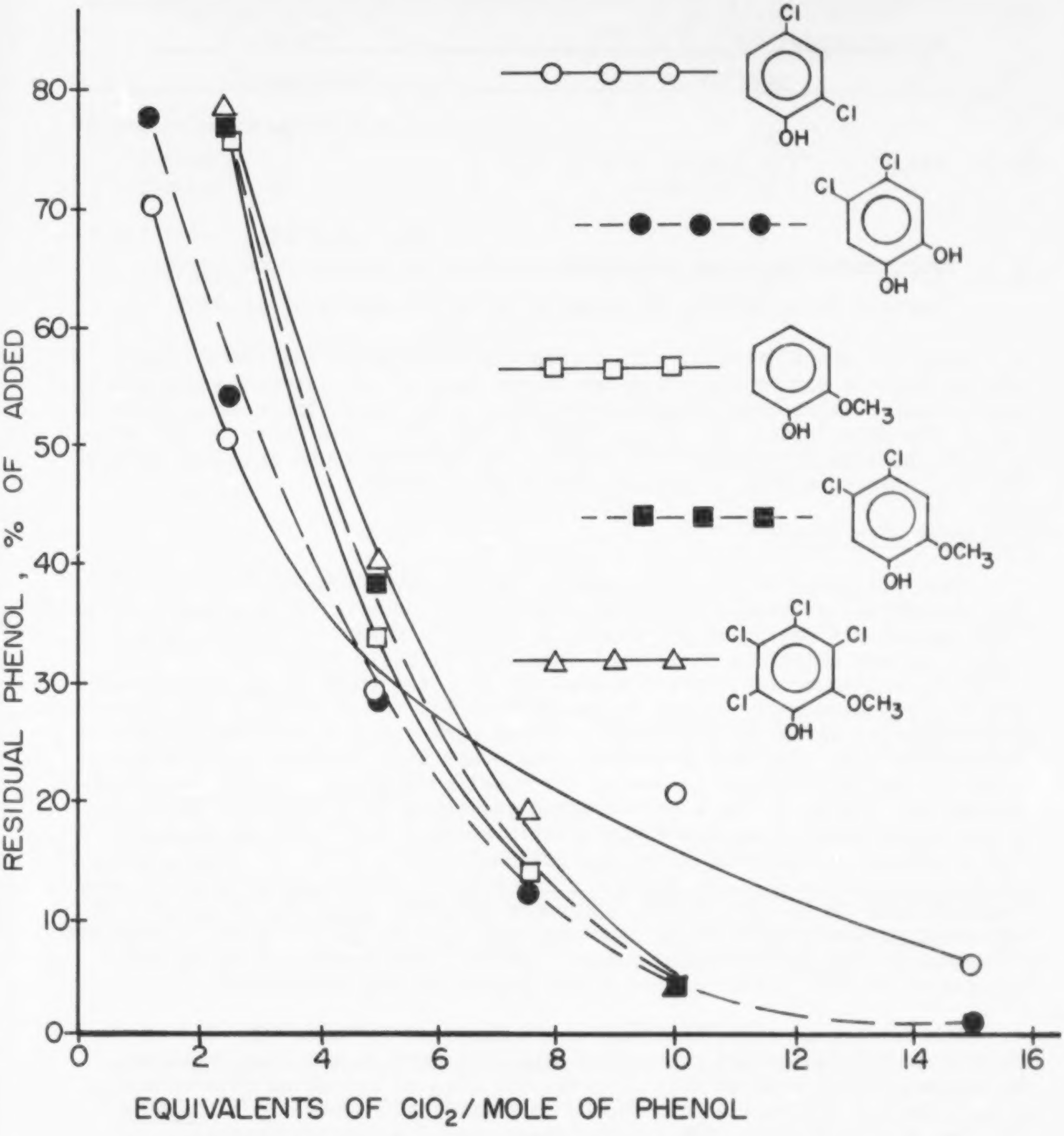


Figure 10. Degradation of selected phenols through reaction with chlorine dioxide.

TABLE 17. CHLORINE DIOXIDE OXIDATION[a] OF TETRACHLOROGUAIACOL-SPENT CAUSTIC EXTRACTION LIQUOR COMBINATIONS

Carbon contributed by tetrachloroguaiacol, % of TOC	Tetrachloroguaiacol removed, %
100	95
30	81
10	58
5	19

[a] Applied 17 mg of ClO_2 to 24 mg of tetrachloroguaiacol at pH 6.

efficiency of phenol removal with increasing dilution by the spent liquor. A similar finding was previously noted in the case of the tetrachloroguaiacol/spent caustic extraction liquor combinations with ozone (Table 16). Comparison of the data in the two tables leads to the conclusion that, in the presence of spent caustic extraction liquor, the selectivity of chlorine dioxide for tetrachloroguaiacol is greater than that of ozone.

Treatment with Alkali

Data presented below and elsewhere (24,36) have demonstrated the detoxification effect of alkali on spent chlorination liquor. In an attempt to relate the detoxification of the liquor to specific phenol or at least phenolic types, aqueous solutions of several phenols previously identified in spent chlorination liquor (23) were prepared and adjusted to pH 11 by addition of alkali and subsequently stored in the dark at 5°C for 24 hours. The appropriate concentrations of the phenols tested were first established by range-finding toxicity studies. A control sample was also prepared and allowed to stand at neutral pH under otherwise identical conditions. During the standing period, the pH of the alkali-treated guaiacols (XXIV and XXV, Table 2), 2,4-dichlorophenol (IV), and 2,4,6-trichlorophenol (VI) remained constant whereas the pH of the alkali-treated catechols (XXI, XXII, and XXIII, Table 2) dropped from 11 to 9.9. At the end of 24 hours, alkali-treated samples were readjusted to pH 7 with 1 N HCl. Standard dilution water was added to each untreated toxicant solution in a volume equal to the total volume of NaOH and HCl added to the corresponding treated sample. All phenol solutions were bioassayed at 14°C using cardiac arrest as the response criterion.

The results of these bioassays are summarized in Table 18. All of the chlorocatechols evidenced pronounced detoxification on alkaline treatment. At concentrations greater than 20 times the LC_{50} of untreated chlorophenol, alkali-treated chlorocatechols caused no significant mortality in *Daphnia*. For the chloroguaiacols, 2,4-dichlorophenol and 2,4,6-trichlorophenol, no significant differences were apparent in the toxicities of the treated and untreated samples. However, with each of these compounds, the toxicity of the treated sample was consistently lower than the untreated sample, with detoxification factors ranging from 0.701 to 0.826.

TABLE 18. EFFECT OF ALKALINE TREATMENT (pH 11) ON THE TOXICITY OF SOME CHLOROPHENOLS TO DAPHNIA MAGNA

Chlorophenol	24 hr LC_{50} (95% confidence interval) in µ moles/liter	g[a]	Δ[b]
4,5-Dichlorocatechol (XXI)			
untreated	6.64 (4.96-7.81)	0.240	<0.037
treated[c]	>178 [0%]	-	
3,4,5-Trichlorocatechol (XXII)			
untreated	3.39 (2.92-3.86)	0.222	<0.055
treated	>62.0 [0%][d]	-	
3,4,5,6-Tetrachlorocatechol (XXIII)			
untreated	2.23 (1.25-2.80)	0.319	<0.039
treated	>57.5 [10%][d]	-	
4,5-Dichloroguaiacol (XIV)			
untreated	98.3 (79.0-135)	0.309	0.826
treated	119 (96.6-189)	0.299	
4,5,6-Trichloroguaiacol (XV)			
untreated	22.0 (15.6-26.5)	0.261	0.701
treated	31.4 (26.3-40.8)	0.242	
2,4-Dichlorophenol (IV)			
untreated	51.9 (40.2-90.1)	0.424	0.761
treated	68.2 (51.2-175)	0.408	
2,4,6-Trichlorophenol (VI)			
untreated	13.2 (11.4-16.0)	0.206	0.811
treated	16.9 (14.8-21.5)	0.276	

[a] As discussed by Finney (11), the "g" value can be used as an index of the quality of the bioassay, i.e., how well the observed mortality pattern corresponds to the best estimate of the probit line. In general, "g" values of 0.4 or less indicate good agreement

[b] LC_{50} of untreated compound LC_{50} of treated compound

[c] Each treatment consisted of addition of NaOH to bring pH to 11.0

[d] Percent mortality at specified concentration

The detoxification of the chlorocatechols (XXI-XXIII) possibly may be associated with loss of organically bound chlorine since structures of this kind have previously been shown to undergo alkali-catalyzed hydrolysis of chloro substituents (19). Conversely, 4,5-dichloroguaiacol (XIV) that sustained a comparatively small loss of organically bound chlorine in the same study, showed a statistically insignificant decrease in acute toxicity (Table 18). This observed relationship does not, however, preclude the involvement of other, as yet unidentified, factors.

DEGRADATION OF CHLOROPHENOLS BY BIOLOGICAL TREATMENT

The biodegradation tests involved addition of individual microorganisms (fungi) and a mixed microbial population to solutions of various phenols; the phenols were identical to those detected in spent chlorination and caustic extraction liquors. Since, in the course of performing the biological treatments, the aqueous phenol solutions were exposed to air throughout the test period, control experiments were conducted in the absence of microorganisms in order to evaluate the effect of aeration alone on the removal of the phenols. In these tests, solutions of the various chlorophenols (conc., 20 ppm) were filtered through a Seitz filter to remove any microorganisms, then shaken on a horizontal shaker for one week at 28°C. The residual phenol was removed by solvent extraction and determined by gas chromatography.

The results of the aeration treatments are shown in Table 19. The extent of phenol removal appears to be related to the structure of the phenolic ring. Thus, both guaiacol derivatives (XIV and XVI) were recovered in the highest yield (70-75 percent), 50 percent of the di- and trichlorophenols (IV and VI, resp.) remained after one week, while less than 5 percent of the catechols survived after the same period. No attempt was made to characterize the phenolic breakdown products, but it was speculated that oxidation of the phenolic rings to quinonoid and acyclic moieties probably occurred, particularly in the case of the catechols (XXI and XXIII). The color developed in the aerated solutions is supportive of the proposed formation of quinonoid units. In addition to oxidation, some hydrolysis of chloro substituents is a likely possibility.

In a somewhat similar investigation, Cain (3) aerated aqueous solutions of several phenols for varying periods of up to 10 days at ambient temperature. Samples were withdrawn at various intervals and the amount of residual phenol measured by ionization difference spectroscopy. The results of this analysis are shown in Table 20. In agreement with the findings reported in Table 19, the catechol derivatives (XVII, XVIII, XX) were degraded more extensively than the guaiacols (IX, XIII). Among the catechols, degradation decreased slightly with increasing numbers of chloro substituents on the phenolic ring. In all instances, the rates of phenol removal were low.

The extents of phenol removal found in the present investigation were slightly greater in the case of the guaiacols and very much greater for the catechols than those indicated by Cain's results after comparable aeration periods. The explanation for this difference may be related to the roughly 4-fold higher phenol concentrations and a slightly lower reaction temperature that Cain used. The analytical methods employed in the two investigations, gas chromatography and ionization difference spectroscopy, may also have contributed to some of the difference.

TABLE 19. EFFECT OF AERATION ON REMOVAL OF CHLOROPHENOLS[a]

Chlorophenols	Residual Chlorophenol, %[b] Aeration alone	 Aeration + fungus[c]
4,5-Dichloroguaiacol (XIV) Tetrachloroguaiacol (XVI)	70-75	55 16
2,4-Dichlorophenol (IV) 2,4,6-Trichlorophenol (VI)	50	15 40
4,5-Dichlorocatechol (XXI) Tetrachlorocatechol (XXII)	<5	-[d] -

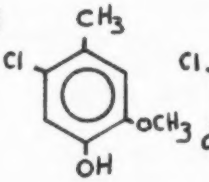
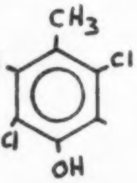
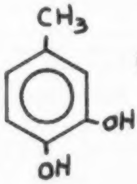
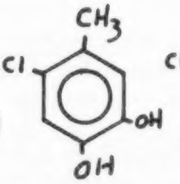
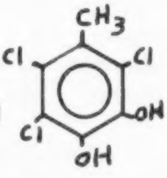
[a] Conc.: 20 ppm; aeration (shaking) time: 1 week; temp., 28°C

[b] Corrected for incomplete recovery by solvent extraction

[c] *Candida utilis* (yeast)

[d] Not determined

TABLE 20. RATE OF REMOVAL OF PHENOLS DURING AERATION

Time, Days	Residual phenol, % Phenol  IX	 XIII	 XVII	 XVIII	 XX
1	99	-	98	100	98
2	98	-	96	94	93
3	98	-	88	88	92
4	97	-	81	85	92
5	96	95	75	83	88
10	-	91	-	-	-

The expectation is that the various phenolic types listed in Tables 19 and 20 will behave in a qualitative manner when subjected to aeration as components of spent chlorination and caustic extraction liquors. However, the rate and degree of their removal in the technical aeration treatment cannot be accurately judged from the data in Tables 19 and 20.

In the few instances where aeration was performed in the presence of a fungus (*Candida utilis*), the amount of phenol removed was increased over that resulting from aeration alone. Under the test conditions, however, chlorophenol removal was incomplete in every instance. In earlier work, Leach, Mueller, and Walden (56) found that tetrachloroguaiacol could be biodegraded by an inoculum of acclimated activated sludge after an induction period of approximately 5 days. The guaiacol was completely consumed after approximately 10 days' fermentation treatment. In other biodegradation tests, three fungi, *Paecilomyces varioti*, *Penicillium variabile*, and *Trichoderma koningii*, were examined for their ability to remove phenolic compounds from a glucose-yeast extract-peptone liquid medium in a one-week incubation period. The amount of residual phenolic compound in the medium was determined by gas chromatography with the results shown in Table 21. The data in this table represent the combined effects of aeration and microorganism attack on phenol breakdown.

The most extensively degraded phenol was 4-methylcatechol (see Table 21) and all three fungi totally removed this compound from the media after one week of incubation. At the other end of the reactivity scale, the veratrole derivatives (XXIV and XXV) proved to be exceptionally resistant to fungal attack. In between these extremes, the fungi evidenced considerable differences in their ability to degrade the same compound.

No consistent correlations were found between toxicity (see Table 3) and susceptibility to biodegradation. Thus, the toxic effect of 2,4,6-trichlorophenol (VI) on *Trichoderma koningii* and *Penicillium variabile* were similar whereas in the biodegradation tests, the former fungi totally removed this phenol from the medium but the latter only to the extent of 50%.

Gas chromatographic analysis of the ethanol extract of the mycelia *Paecilomyces varioti* following incubation indicated that the fungal mycelia had not removed the phenols from the media by physical adsorption. The removal of phenolic compounds from the media can therefore be attributed to fungal attack, coupled with aeration effects.

Cain (3) examined the biodegradation of many of the phenols listed in Table 2 by the mixed microbial population present in the sludge of a paper mill waste treatment plant. In these tests, solutions of the phenols were inoculated with diluted samples of the sludge and incubated at ambient temperatures for periods up to 15 days. Residual phenol content was determined at selected reaction intervals using gas chromatography.

TABLE 21. BIODEGRADATION OF PHENOLS BY FUNGI IN LIQUID CULTURE MEDIA

Phenol		Initial conc., ppm	Residual phenol, %		
			Paecilomyces varioti	*Trichoderma koningii*	*Penicillium variabile*
Creosol	VII	50	70	62	1.4
6-Chlorocreosol	VIII	50	82	70	79
5-Chlorocreosol	IX	50	75	68	83
5,6-Dichlorocreosol	X	10	94	75	62
3,6-Dichlorocreosol	XI	10	89	6.8	6.6
3,5-Dichlorocreosol	XII	10	70	34	16
Trichlorocreosol	XIII	10	77	0	0
4-Methylcatechol	XVII	50	0	0	0
4-Methyl-5-chlorocatechol	XVIII	50	70	44	61
4-Methyl-5,6-dichlorocatechol	XIX	10	87	87	11
4-Methyl-3,5-6-trichlorocatechol	XX	10	40	50	44
4-Methylveratrole	XXIV	50	81	89	94
4-Methyl-6-chloroveratrole	XXV	50	83	95	100
p-Cresol	I	50	90	60	66
4-Methyl-2,6-dichlorophenol	V	10	53	6.0	3.0
2,4,6-Trichlorophenol	VI	10	67	0	52

All of the phenols examined by Cain were at least partially degraded under the test conditions. The rate and extent of phenol removal was found to vary widely from compound to compound and consistent trends correlating structure with these two parameters were not apparent. As in the previously described tests employing individual fungi, the biodegradation with microorganisms in the sludge was performed in concert with aeration. The measured decrease in phenol content was therefore, in all probability, a composite of the effects of biological and chemical processes. Failure to perceive clear-cut trends in Cain's data may therefore be due to their being masked as a result of interference on the part of one or the other type of process.

In view of the widespread use of biological processes in the treatment of pulping and bleaching wastes, additional research aimed at optimization of the operating parameters affecting phenol and chlorophenol breakdown would appear to be highly desirable.

CHEMICAL TREATMENT OF SPENT CHLORINATION AND CAUSTIC EXTRACTION LIQUORS

In these treatments, the chemical was generally applied to either of the two spent liquors but rarely to both. The choice of liquor to be used in any given treatment was dictated by pH considerations; thus, those chemicals known to be effective under alkaline conditions were applied to spent caustic extraction liquor whereas those functioning in the desired manner in acidic media were added to the spent chlorination liquor.

In the application of those chemicals (oxidants) used as pulp bleaching agents, the treatment temperatures selected coincided with those used in the bleaching process. The treatments were continued until the chemical was completely consumed in order to avoid its contributing to acute toxicity in any subsequent bioassays.

Chlorination Treatment

Spent chlorination liquor was reacted with solutions of chlorine water at room temperature without pH adjustment. In one treatment, 0.2 mg Cl_2/ml spent liquor was consumed with a corresponding 35% reduction of phenol content as indicated by ionization difference spectra measurement. In a second treatment, 0.8 mg Cl_2/ml spent liquor was consumed with an accompanying loss of 70% of the phenol content. The reacted solutions were subjected to bioassay using Daphnia magna as test organism. In both cases, a distinct increase in toxicity was observed over that found for the untreated liquor. The toxicity of the two treated liquors was not significantly different.

Results reported for the phenol contents of spent chlorination and caustic extraction liquors here and elsewhere in this report must be regarded as only approximate. This stems partly from the fact that the absorptivity values of the component phenols of the liquor vary substantially among themselves. Thus, any attempt to calculate phenol concentration must involve the use of an "average" absorptivity value which cannot be determined accurately. Hence, the values for phenol content listed throughout this report indicate "phenol" content changes resulting from a specified treatment. Even this usage is suspect since the alkali used to effect ionization may induce other chemical changes in the molecule that affect its spectral characteristics.

Hypochlorous Acid Treatment

The pH of samples of chlorination liquor was adjusted to 4.75 and buffered with acetate to obtain a high concentration of hypochlorous acid after the addition of chlorine. In two treatments, 0.2 and 0.37 mg of HOCl/ml of spent liquor were consumed, which resulted in the loss of 35 and 82% of the phenols, respectively, as indicated by ionization ultraviolet spectroscopy. As in the above instance, the bioassay test results indicated an increase in toxicity as a result of the oxidation with hypochlorous acid.

Sodium Hypochlorite Treatment

A sample of SCEL was reacted with 0.7 mg of NaOCl/ml of liquor at 60°C. After three hours, the oxidant was completely consumed and the phenol content was reduced to 49%. With a larger application of hypochlorite (2.0 mg/ml of liquor), consumption at the same temperature was complete in nine hours and phenol removal was 95% complete.

After these tests had been completed, it was discovered that the commercial sample of sodium hypochlorite used had a high chloride concentration that which could have affected the LC_{50} values of the bioassay. Accordingly, another experiment was performed with laboratory-prepared hypochlorite (7.8 mg NaOCl/ml liquor, 60°C, reacted to exhaustion (~24 hrs)) having an acceptably low chloride content. The acute toxicity of the hypochlorite-treated liquors was determined using *Daphnia magna* and the results are reported in Table 22. As in the previous instances where chlorine and hypochlorous acid were applied, the trend shown by the data is that hypochlorite treatment actually resulted in an increase in toxicity.

TABLE 22. EFFECT TO *DAPHNIA MAGNA* OF SODIUM HYPOCHLORITE TREATMENT ON THE ACUTE TOXICITY OF SPENT CAUSTIC EXTRACTION LIQUOR

Liquor	Exposure time, hrs.	LC_{50} in ppm TOC (95% C.I.)
Original	24	440 (412-469)
	96	367 (286-490)
NaOCl-Treated		
0.7 mg/ml liquor	24	442 (400-489)
	96	271 (110-668)
2.0 mg/ml liquor	24	250 (200-314)
	96	155 (122-196)
7.8 mg/ml liquor	24	294 -[a]
	48	281 -[a]

[a] Not determined

The explanation for the increase in toxicity of the spent liquors as a consequence of the three aforementioned treatments is not readily apparent. One possibility is that the treatments resulted in an increase in the amount of organically bound chlorine, particularly that substituted on the aromatic ring. The formation of non-phenolic oxidation products more toxic than the phenols themselves is also feasible, especially in the case of reaction with hypochlorite. Generally speaking, however, continued oxidation of chloro-organic compounds to smaller fragments ultimately would be expected to have a beneficial effect with respect to toxicity reduction since organically bound chlorine would be converted to chloride in the process.

Treatment of Spent Chlorination Liquor with Chlorine Dioxide

Spent chlorination liquor was reacted with chlorine dioxide (0.27 meq/ml of liquor) at room temperature until the oxidant was exhausted (∿5 hours). The pH of the reaction mixture remained at 2 or lower during the treatment. The 24- and 48-hr LC_{50} values for the resulting liquor were 153 and 75 ppm TOC, respectively, using Daphnia magna as the test organism. When compared to the value for the original liquor (see Table 7), the results indicate that a modest amount of detoxification had resulted.

Treatment of Spent Caustic Extraction Liquor with Hydrogen Peroxide

Spent caustic extraction liquor was buffered (∿pH 10.5) and stabilized with sodium silicate and magnesium sulfate, respectively, and reacted with hydrogen peroxide (4 meq of H_2O_2/150 ml of liquor) at 70°C. Consumption of peroxide under these relatively severe conditions was very slow and was only 50% complete after three hours' reaction. Complete consumption and/or decomposition of peroxide was achieved only after ∿24 hours of heating.

The 24- and 48-hr LC_{50} values for the peroxide-treated liquor were 202 and 133 (ppm of TOC), respectively, when tested with Daphnia magna. Comparison of these values with those of the untreated SCEL (Table 7) reveals that the peroxide treatment actually produced an increase in acute toxicity. This finding agrees with results reported by Betts and Wilson (57). Although additional tests should be performed to confirm this trend, the comparative unreactivity of the peroxide toward the spent caustic extraction liquor, even at elevated temperature, prompts the conclusion that such a process would not be technically feasible in any event.

Treatment of Spent Chlorination and Caustic Extraction Liquors with Ozone

Spent chlorination liquor was ozonized, both at its ambient pH (∿2) and at pH 8, while spent caustic extraction liquor was reacted with ozone only at pH 8. Acute toxicity tests were performed on the ozonized liquors with Daphnia magna and brine shrimp (Artemia salina). The results are summarized in Table 23.

The ozonization consumption patterns (cf. Table 23) for the three liquor samples are similar and consist of an essentially quantitative uptake of ozone in the initial stage of the treatment, followed by an ever-diminishing

TABLE 23. CHARACTERISTICS OF ORIGINAL AND OZONIZED SPENT CHLORINATION AND CAUSTIC EXTRACTION LIQUORS

Ozone, mg/ml of liquor						Acute toxicity	
Applied	Consumed	TOC, ppm	Color (color units)	"Phenol" removal, %	Org. bound chlorine, ppm	24-Hr LC_{50} in ppm TOC (95% C.I.)	
			Spent Chlorination Liquor (pH 2)				
						Brine Shrimp	Daphnia magna
0	0	268	1,383	0	91	342 (126-[a])	107 (81.2-212)
0.052	0.034	254	917	(-)4	69	-	-
0.208	0.121	214	575	7	67	-	-
0.521	0.145	204	429	55	16	284 (76-[a])	53.2 (40.3-72.3)
			Spent Chlorination Liquor (pH 8)				
0	0	268	1,383	0	91	-	-
0.053	0.053	261	933	17	61	-	-
0.213	0.193	237	370	18	31	-	-
0.532	0.296	195	200	34	34	26 (19-33)	-
			Spent Caustic Extraction Liquor (pH 8)				
0	0	1640	18,290	0	209	1323 (466-[a])	-
0.087	0.086	1480	9,458	36	133	-	-
0.232	0.175	1360	2,790	36	47	304 (238-434)	-

[a] No meaningful estimate due to low mortality

consumption with continuing introduction of ozone at a constant flow rate. Under the same conditions, the total organic carbon (TOC) decreased in each instance, but the total decrease observed within the test limits was only in the range 17-27%. Thus, a sizable portion of the spent liquor constituents resisted degradation to volatile carbon-containing compounds even in the presence of excess ozone.

A decrease in the color of the spent liquors accompanied the ozone treatment as has been reported and commented on previously (58,59). The overall decrease in color was greater when the ozonization was performed in an alkaline medium.

The initial and residual phenol contents of the spent liquors were measured by means of ionization difference spectroscopy. Because of the errors inherent in this method when applied to complex mixtures, the data for residual phenol content in Table 23 have to be viewed with some degree of skepticism. Of particular interest with regard to these data is the apparent increase in "phenol" content observed in the case of SCL (pH 2) in the initial stages of ozonization. The obvious implication of this finding, which has been substantiated in replicate tests, is that either additional ionizable groups (including phenolic hydroxyls) were created as a consequence of the ozonization treatment or that pre-existing structures were chemically modified in such a way that their Δ absorbance values were increased. Although the exact extent of phenol removal is open to question, the overall sense of the data in Table 23 is that it decreases substantially in each instance.

An increase in chloride ion content or a decrease in organically bound chlorine content of ozonized chloro-organic material can be considered indicative of the extent of oxidative breakdown. In the case of the spent liquors, the organically bound chlorine content (Table 23) is seen to decrease with increasing consumption of ozone in all but one instance. In all cases, however, some chlorine-containing residues remain at the conclusion of the treatment. The possibility that extended ozone treatment of the liquors would result in complete removal of organically bound chlorine seems unlikely in view of the observed diminished reactivity of the residual material toward ozone. Thus, spent liquors initially either contain chlorine-substituted structures resistant to ozone or are transformed to such during breakdown by ozone.

The results of the acute toxicity test for the spent chlorination liquor (pH 2) using Daphnia magna and brine shrimp as the test organisms are somewhat surprising in that the ozonized liquor proved to be equally or slightly more toxic than the untreated sample. An earlier study of Betts and Wilson (57) showed that ozonization of spent chlorination liquor was ineffective for reducing the acute toxicity toward salmon. Other data, collected in the current investigation but not included on Table 23, point to a trend in which a small decrease in acute toxicity appears with small applications of ozone but thereafter increases with increasing ozone dosages. These data are not statistically reliable, however, and hence cannot be interpreted with any degree of confidence.

Because of buffer salts used to control pH in the alkaline ozonization treatments, Daphnia magna was replaced by brine shrimp as the test organism in these cases. The LC_{50} values listed in Table 23 show that ozonization of spent chlorination and caustic extraction liquors in an alkaline (pH 8) condition resulted in a decided increase in toxicity under the applied treatment conditions. (Although no control data are available for a sample of spent chlorination liquor adjusted to pH 8, results cited below show that alkaline treatment of such liquors actually causes a substantial reduction in acute toxicity.)

Viewed collectively, the results in Table 23 relating to acute toxicity lead to the conclusion that in no instance does ozonization cause a substantial reduction in the toxicity of spent chlorination and caustic extraction liquors; and under alkaline ozonization conditions, a decided increase is produced. The reason for the failure of ozone to produce the expected beneficial effect is not obvious, particularly in view of the results reported previously; showing phenols of the types found in spent chlorination and caustic extraction liquors to be extensively degraded by this oxidant. However, the complexity of the spent bleaching liquors with respect to component composition is a factor to be reckoned with in attempting to rationalize the effect of ozonization on their toxicity. It is conceivable, therefore, that the gain in toxicity reduction resulting from the breakdown of phenolic substances by ozone could be offset by the formation of toxic materials or as a result of interactions between the liquor components as described by Durkin (24).

Treatment of Spent Caustic Extraction Liquor (SCEL) with Aluminum Sulfate

Aluminum sulfate (1.5 g) was added to SCEL (220 ml) and allowed to react for one hour at room temperature (pH 5.5). The precipitate was removed by centrifugation, dissolved by addition of HCl, and subsequently purified by dialysis through cellophane. The supernate from the centrifugation and the retentate from the dialysis were adjusted to the volume of the original test sample, tested for phenol content, and bioassayed for acute toxicity with the results shown in Table 24.

The results show that, although the treatment with alum removed a considerable amount of the material in the liquor, a major part of the toxicity initially present was still retained in the supernate. This finding accords with the concept that the aluminum coagulates the higher molecular weight components of the liquor that appear to be less toxic than the lower molecular weight species.

Treatment of Spent Caustic Extraction Liquor (SCEL) with Lime

Sufficient $Ca(OH)_2$ was added to spent caustic extraction liquor to provide a final concentration of 15 g/l. After standing one hour, the precipitate was removed by filtration and the pH of the filtrate was adjusted to 9.4 by the introduction of CO_2. The lime-treated liquor, after removal of $CaCC_3$, had the characteristics recorded in Table 25.

TABLE 24. PROPERTIES OF ALUM-TREATED SCEL

	Original liquor	Supernate	Dialysis retentate
TOC, ppm	1362	360	819
Phenol content, % of original	100	12	68
LC_{50} (24-hr test), ppm	425	145	653
TOC/LC_{50} (toxic units)	3.2	2.5	1.3

TABLE 25. EFFECT OF LIME TREATMENT ON SPENT CAUSTIC EXTRACTION LIQUOR (SCEL)

	Original SCEL	Lime-treated SCEL[a]
TOC, ppm	1630	493[b]
pH	12.4	9.4
Absorbance at 280 nm[b]	44.28	13.6
Absorbance at 400 nm[b]	9.98	2.01
Ether extract		
TOC[b], ppm	192	141
Organically bound chlorine[c], ppm	26.5	15.8
LC_{50}, ppm of TOC) 24-hr test	350	199
LC_{50}, % (v/v)[c]) 24-hr test	21.5	40.4
Toxic units	4.7	2.5
Toxic units, % of original	100	53

[a] After carbonation to pH 9.4

[b] Measured at pH 7.0 in phosphate buffer

[c] Based on original volume

As shown by the data in this table, 80% of the color of the SCEL was removed by lime treatment based on the absorbance decrease at 400 nm. The absorbance at 280 nm was reduced to 30% of its original value, which is approximately the same as the decrease in total organic carbon (TOC). The yield of ether extract expressed as TOC indicates that approximately a 30% reduction resulted from the lime treatment. The organically bound chlorine content was reduced by about 40% by the same treatment.

The LC_{50} value for the lime-treated liquor is lower than that of the original liquor. This means that the residual organic material in the lime-treated liquor was more toxic than that in the original liquor. In other words, the non-toxic fraction was removed in preference to the toxic fraction in the lime treatment. This observation is consistent with the belief that the lime preferentially precipitates the higher molecular weight material which, as was demonstrated in an earlier section, is less toxic than lower molecular weight material. The total toxicity of the SCEL, however, was decreased about 47% when calculated on a toxic unit basis.

Treatment of Lime-Treated SCEL with Activated Carbon and Various Oxidants

The goal was to establish methods that would use a relatively inexpensive chemical (lime) to remove a large fraction of the dissolved material in SCEL, thereby necessitating the use of much smaller quantities of more expensive chemicals to achieve the desired effluent quality. The tests described below were performed.

Adsorption of Lime-Treated SCEL on Activated Carbon--

The adsorption treatments were performed at room temperature for an 18-hr period; samples of spent caustic extraction liquor, with pH values of 5.0 and 9.4, were used. The results are shown in Table 26 and Figure 11. From these results, it is obvious that pH 5.0 is superior to pH 9.4 as an initial test level, both with respect to color and TOC removal at a given application of carbon. The amount of ether-extractable material showed a further decrease (cf. Table 25) and the extract of the pH 5.0 liquor had very little or no organically bound chlorine.

TABLE 26. PROPERTIES OF LIME-TREATED SCEL AFTER REACTION WITH ACTIVATED CARBON

Initial pH	5.0			9.4		
Carbon conc., g/l	5	10	15	5	10	15
Final pH	6.4	6.3	6.3	9.1	9.1	8.8
TOC, ppm	213	213	200	340	302	262
Absorbance at 280 nm	1.91	1.03	0.90	7.30	6.29	4.79
Absorbance at 400 nm	0.08	0.03	0.02	0.88	0.50	0.75
Ether extract						
TOC, ppm[a]	34	32	30	64	53	51
Organically bound chlorine, ppm[a]	1.1	0.0	0.0	5.7	4.0	3.8

[a] Based on original volume

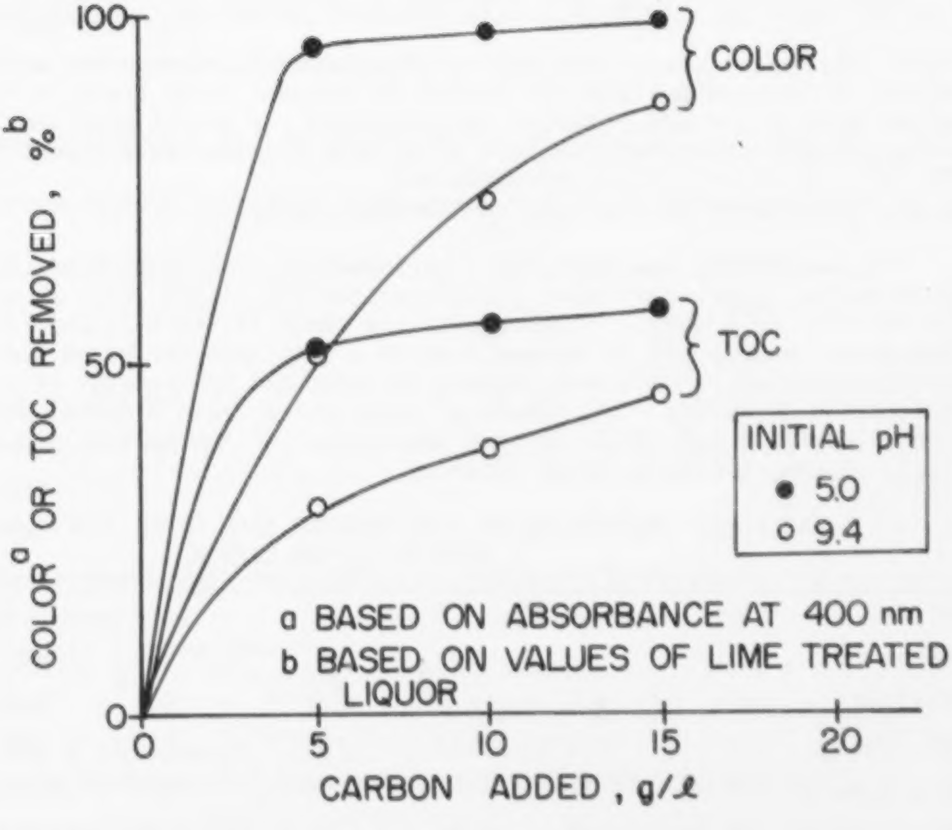


Figure 11. Effect of carbon treatment on the color and TOC of lime-treated spent caustic extraction liquor.

As shown by tests 2 and 3 in Table 27, the liquor samples treated at pH 5.0 and 9.4 showed the same residual toxicity of 30%, thereby indicating that pH had little or no effect on the reduction of this property. Since the LC_{50} values of these same liquors were 150 and 245 ppm of TOC, respectively, it is evident that a greater preference for the removal of non-toxic material existed at pH 5.0 than at pH 9.4.

The carbon dosage of 15 g/l at pH 5.0 (Table 27, test 4) produced essentially the same reduction of toxicity as the 5.0 g/l application of carbon. It seems, therefore, that about 30% of the toxicity is difficult to remove with activated carbon, even when excessive amounts of the latter are applied. If this does indeed prove to be the case, then it may be concluded that, similar to the results of other treatments such as lime precipitation and alum coagulation, activated carbon is limited with respect to its ability to effect 100% removal of toxicity.

A sample of SCEL (diluted one third) was treated directly with activated carbon in order to compare its detoxification effect with that of lime. Comparison of the results (test 1, Table 27) and (test 7, Table 28) clearly reveals the greater effectiveness of lime for toxicity removal. The implication of this finding is that lime removed a greater amount of low molecular substances from the spent liquor than did activated carbon. Failure of activated carbon to effect a somewhat greater reduction in toxicity is somewhat surprising in view of the fact that Betts and Wilson (57) have reported the successful detoxification of spent chlorination liquor on treatment with charcoal.

The small carbon particles that passed through the filter paper when the carbon was filtered from the treated SCEL were removed by filtration through a Millipore (5.0 μ) membrane. As shown by the results of tests 8 and 9 (Table 28), there is little difference in toxicity removal with and without the membrane treatment, thereby excluding the possibility that residual carbon particles contributed significantly to toxicity. The above finding also suggests that the residual toxicity of liquors corresponding to tests 2, 3 and 4 in Table 27 was not caused by the presence of minute quantities of activated carbon particles.

Oxidation of Lime-Treated Liquor with Ozone, Chlorine Dioxide, and Hydrogen Peroxide--

For the purpose of reducing the number of samples subjected to bioassay, the properties of the lime-treated SCEL were first determined as a basis for the screening process. The results of these analyses are reported in Tables 29, 30 and 31. The results for carbon-treated liquor (Table 28) provide a reference for comparison.

As an initial pH value, 5.0 gave better results with respect to TOC removal in the cases of ozone and chlorine dioxide whereas the opposite was true for hydrogen peroxide. TOC removals were in all instances under 25% based on that of the lime-treated liquor. As shown by the plot in Figure 11,

TABLE 27. ACUTE TOXICITY OF SCEL TREATED SEQUENTIALLY WITH LIME AND ACTIVATED CARBON

Test No.	Conc. of act. carbon, g/l	Initial pH	TOC, ppm	24-hr LC_{50}		Toxic units	Residual toxic units, %
				ppm of TOC	% (v/v)		
1	0	9.4	493	199	40.4	2.5	53
2	5	5.0	213	150	72.5	1.4	30
3	5	9.4	340	245	72.5	1.4	30
4	15	5.0	200	132	65.8	1.5	32

TABLE 28. ACUTE TOXICITY OF CARBON-TREATED SCEL[a]

Test No.	Conc. of act. carbon, g/l	Initial pH	TOC, ppm	24-hr LC_{50}			Toxic units	Residual toxic units, %
				ppm of TOC	% (v/v)	% (v/v)[b]		
5	0	9.4	543	351	64.7	21.6	4.6	(100)
6	0	9.4	543	348	64.0	21.3	4.7	
7	5	5.0	-[c]	-	87.5	29.2	3.4	74
8	15	5.0	-	-	84.3	28.1	3.6	78
9[d]	15	5.0	-	-	85.5	28.5	3.5	76

[a] Diluted to 1/3 its original volume

[b] Based on original (i.e., undiluted) volume of SCEL

[c] Not determined

[d] Filtered through Millipore filter

TABLE 29. EFFECT OF OZONE OXIDATION ON THE PROPERTIES OF LIME-TREATED SCEL

Initial pH	5.0			9.4		
Ozone added, meq/l	56	92	108	50	98	150
Ozone residual, meq/l	0	0	0	0	0	0
Final pH	5.3	5.5	5.6	9.1	9.1	9.0
TOC, ppm	370	357	359	508	480	468
Absorbance at 280 nm	4.12	2.52	2.30	-[a]	-	-
Absorbance at 400 nm	0.85	0.45	0.36	1.02	0.52	0.33
Ether extract						
TOC, ppm[b]	54	74	41	68	45	56
Organically bound chlorine, ppm[b]	5.1	3.2	2.3	8.5	5.8	3.6

[a] Not determined

[b] Based on original volume

TABLE 30. EFFECT OF CHLORINE DIOXIDE ON THE PROPERTIES OF LIME-TREATED SCEL

Initial pH	5.0			9.4		
ClO_2 added, meq/l	47	86	137	30	52	126
ClO_2 residual, meq/l	0	33	87	10	18	42
Final pH	3.2	2.5	2.5	8.7	7.8	7.0
TOC, ppm	440	384	359	495	457	359
Absorbance at 280 nm	2.11	2.42	2.51	-[a]	-	-
Absorbance at 400 nm	0.20	0.21	0.13	-	-	-
Ether extract						
TOC, ppm[b]	57	39	35	141	138	139
Organically bound chlorine, ppm[b]	5.2	7.7	6.4	24.0	18.0	15.9

[a] Not determined

[b] Based on original volume

TABLE 31. EFFECT OF HYDROGEN PEROXIDE OXIDATION ON THE PROPERTIES OF LIME-TREATED SPENT CAUSTIC EXTRACTION LIQUOR

Initial pH	5.0			9.4		
H_2O_2 added, meq/l	118	235	353	24	59	118
H_2O_2 residual, meq/l	119	238	197	0	4	8
Final pH	5.3	5.6	5.1	9.0	9.1	8.9
TOC, ppm	479	489	451	453	413	373
Absorbance at 280 nm[a]	9.18	8.40	8.14	9.95	9.95	7.95
Absorbance at 400 nm[a]	1.15	1.03	0.99	0.99	0.90	0.69
Ether extract						
TOC, ppm	129	92	102	89	97	84
Organically bound chlorine, ppm	14.6	12.8	14.7	11.9	9.5	9.0

[a] Based on original volume

carbon treatment resulted in the removal of more than 50% of TOC. This large difference may be due to the fact that the oxidation products are mainly organic acids that still contribute to TOC, whereas in the carbon treatment, some of these acids are actually removed from solution by adsorption onto the carbon surface. The values for the other parameters were reduced more than that of the TOC but less than that observed after treatment with activated carbon at pH 5.0.

Of the three oxidants applied to the lime-treated spent caustic extraction liquor, only the ozone was totally consumed (oxidation and/or decomposition) in a reasonable length of time at room temperature. The sluggishness of the reactions of chlorine dioxide and hydrogen peroxide with the lime-treated liquor necessitated these reaction mixtures being heated for an extensive period of time in order to exhaust the oxidant. Complete consumption of oxidant was imperative since residual amounts of peroxide and chlorine dioxide (particularly the latter) were found to have a considerable positive effect in increasing acute toxicity.

The values shown in Table 32 reveal that of the three oxidants tested, toxicity reduction was effected only with ozone and hydrogen peroxide. The benefits provided by treatment with these reagents were fairly modest, however, in comparison to the toxicity reduction resulting from the initial treatment of the spent caustic extraction liquor with lime. Contrary to the benefits accruing from the use of peroxide and ozone, chlorine dioxide treatment actually increased the acute toxicity of the lime-treated liquor. As noted previously, chlorine dioxide application to whole spent chlorination liquor produced a small reduction in acute toxicity.

TABLE 32. ACUTE TOXICITY OF LIME-TREATED CAUSTIC EXTRACTION LIQUOR REACTED WITH OZONE, CHLORINE DIOXIDE, AND HYDROGEN PEROXIDE

Test No.	Oxidant	Conc. of oxidant, meq/l	Initial pH	TOC, ppm	24-hr LC_{50} TOC, ppm	24-hr LC_{50} % (v/v)	Toxic Units	Residual toxic units, %
-	-	-	(SCEL)	1630	350	21.5	4.7	100
-	-	-	(Lime-Treated SCEL)	493	199	40.4	2.5	53
10	O_3	50	5.0	370	204	55.1	1.8	39
11	ClO_2[a]	50	5.0	409	61	14.9	6.7	143
12	H_2O_2[b]	50	9.4	434	281	64.8	1.5	32

[a] Heated at 78°C for 12 hrs after treatment

[b] Heated at 78°C for 4.5 hrs after treatment

The amounts of chlorine dioxide, ozone and peroxide applied to the lime-treated liquor were equivalent to the addition of 6.1, 10.8, and 7.7 g/kg of pulp, respectively, in a hypothetical bleaching stage in which pulp consistency was 10%. Thus, even in those instances where modest reductions in acute toxicity were achieved, the cost in terms of expenditure of chemical would have to be considered economically prohibitive and it is concluded that none of the above-described combination processes is likely to have much of a future as a technical approach to waste treatment.

Treatment of Spent Chlorination Liquor with Sodium Hydroxide

The pH of SCL was adjusted to various pH levels and maintained at those levels throughout the test by the addition of aqueous sodium hydroxide. After a predetermined reaction period at room temperature, the phenol and organically bound chlorine contents of the solutions were monitored. The results are shown in Tables 33 and 34.

TABLE 33. EFFECT OF ALKALI TREATMENT OF SPENT CHLORINATION LIQUOR ON PHENOL AND ORGANICALLY BOUND CHLORINE CONTENTS

pH	Phenol loss, %	Loss of organically bound chlorine, % 16 hours	12 days
2	0	0	18
5	0	11	36
6	7	-	-
7	33	14	44
8	42	-	-
9	51	19	55
10	69	38	74
11	80	65	75
12	73	-	-
13	∿67	-	-

The phenol loss was difficult to quantify accurately due to a shift in the absorption maximum from 290 to 310-320 nm in the ionization difference spectrum. Nevertheless, the results clearly show a large decrease in phenol content, particularly in the pH 7-10 range. The loss in phenol content was paralleled by a decrease in organically bound chlorine, which was time dependent. The maximum values for the loss of phenol and organically bound chlorine were identical (∿75%) and might be related although this was not established.

TABLE 34. RATE OF REMOVAL OF ORGANICALLY BOUND CHLORINE FROM SPENT CHLORINATION LIQUOR WITH INCREASING pH

	Loss of organically bound chlorine, %		
pH	ay 1	ay 9	Day 12
2	<1	15	18
5	11	-	36
7	14	-	44
9	19	53	55
10	38	72	74
11	65	75	75

The effect of pH on the rate of removal of organically bound chlorine from spent chlorination liquor is revealed by the data in Table 34. The trend for enhancement of the rate with increase in pH is clearly evident, especially in the pH 9-10 interval. The maximum loss of chlorine appears to be reached in shorter periods as the pH is raised.

In tests designed to determine the effect of an alkaline treatment on acute toxicity, the pH of samples of spent chlorination liquor (SCL) was adjusted to 7, 9, 10 and 11 by the addition of sodium hydroxide solution and allowed to stand in the dark for 24 hours at 5°C. The above samples and an untreated pH 2 SCL control were bioassayed after the 24-hour period using Daphnia magna as the test organism.

The effect of these alkaline treatments, as indicated by the data in Table 35, was to cause a reduction in the acute toxicity of SCL. The amount of toxicity reduction increased with increasing treatment pH. The beneficial effect of alkali with respect to toxicity reduction is in agreement with the results of an earlier investigation reported by Leach et al. (36) who detoxified spent chlorination liquor with combined aeration and alkaline treatment over a six-day period.

Several of the bioassays reported in Table 35, especially corresponding to the higher pH treatment levels, gave unsatisfactorily high g values. In all cases, this is attributable to low response rates even at the highest concentration tested (100%, 218 ppm TOC) and is not the result of heterogeneous concentration-response patterns.

Detoxification values, Δ, were calculated as the ratio of the EC_{50} (immobility as the response criterion) at pH 2 for a given exposure period divided by the EC_{50} of the alkaline treated sample for the same exposure period. These values suggest a consistent decrease in potency with increasing pH as is illustrated in Figure 12. Although good multiple estimates of potency over a wide pH range are available only for 48-hour exposure periods, these data suggest that the relationship between detoxification and treatment pH is linear.

TABLE 35. EFFECT OF ALKALI ON THE TOXICITY OF SPENT CHLORINATION LIQUOR TO DAPHNIA MAGNA

Treatment pH	Exposure period	EC_{50} (95% confidence interval) in ppm TOC	Δ[a]	g
pH 2	24 hrs.	95.3 (53.6 - 169)	1.00	1.270
	48 hrs.	37.5 (14.5 - 97.7)	1.00	0.518
	96 hrs.	<41.4 {80%} [c]	1.00	-
pH 7[b]	24 hrs.	117 (91.8 - 150)	0.80	0.219
	48 hrs.	51.0 (24.6 - 106)	0.84	0.550
	120 hrs.	41.4 (26.8 - 63.9)	0.91	0.438
pH 9	24 hrs.	338 (72.2 - 1571)	0.28	1.01
	48 hrs.	85.4 (60.0 - 122)	0.50	0.386
	96 hrs.	64.5 (42.7 - 97.7)	<0.64	0.255
pH 10	24 hrs.	>218 {10%}[c]	<0.43	-
	48 hrs.	152 (56.2 - 412)	0.28	2.37
	96 hrs.	146 (37.9 - 567)	<0.28	2.97
pH 11	24 hrs.	>218 {0%}[c]	<0.43	-
	48 hrs.	>218 {0%}[c]	<0.22	-
	120 hrs.	534 (92.7 - 3075)	0.07	1.41
pH 11	24 hrs.	>218 {10%}[c]	<0.43	-
	48 hrs.	233 (90.3 - 604)	0.16	0.565
	96 hrs.	233 (90.3 - 604)	<0.18	0.565

[a] EC_{50} of pH 2 liquor for given exposure period ÷ LC_{50} of liquor treated at specified pH for the corresponding exposure period.

[b] pH initially adjusted to specified value and not thereafter controlled; in all other instances, alkali was periodically added to restore pH to initial value.

[c] % mortality at specified concentration.

In the course of the alkaline treatment of spent chlorination liquor, the pH fell below the prescribed level, seemingly as the result of the hydrolysis of organically bound chlorine. In one bioassay, the pH was periodically readjusted back to the initial value (pH 11) by addition of alkali whereas, in another test, the pH was not readjusted. The results in Table 35 (last two tests) indicate that this difference in test procedure did not have a statistically significant effect on toxicity.

The effect of prolonged (two week) treatment of spent chlorination liquor at pH 7 is summarized in Table 36. For EC_{50}'s at 48 and 96 hours, this treatment resulted in a statistically significant decrease in toxicity. Comparing the potency values in Table 36 with the pattern in Figure 12, treatment at pH 7 for two weeks appears to have about the same detoxification effect as treatment at pH 10 for 24 hours.

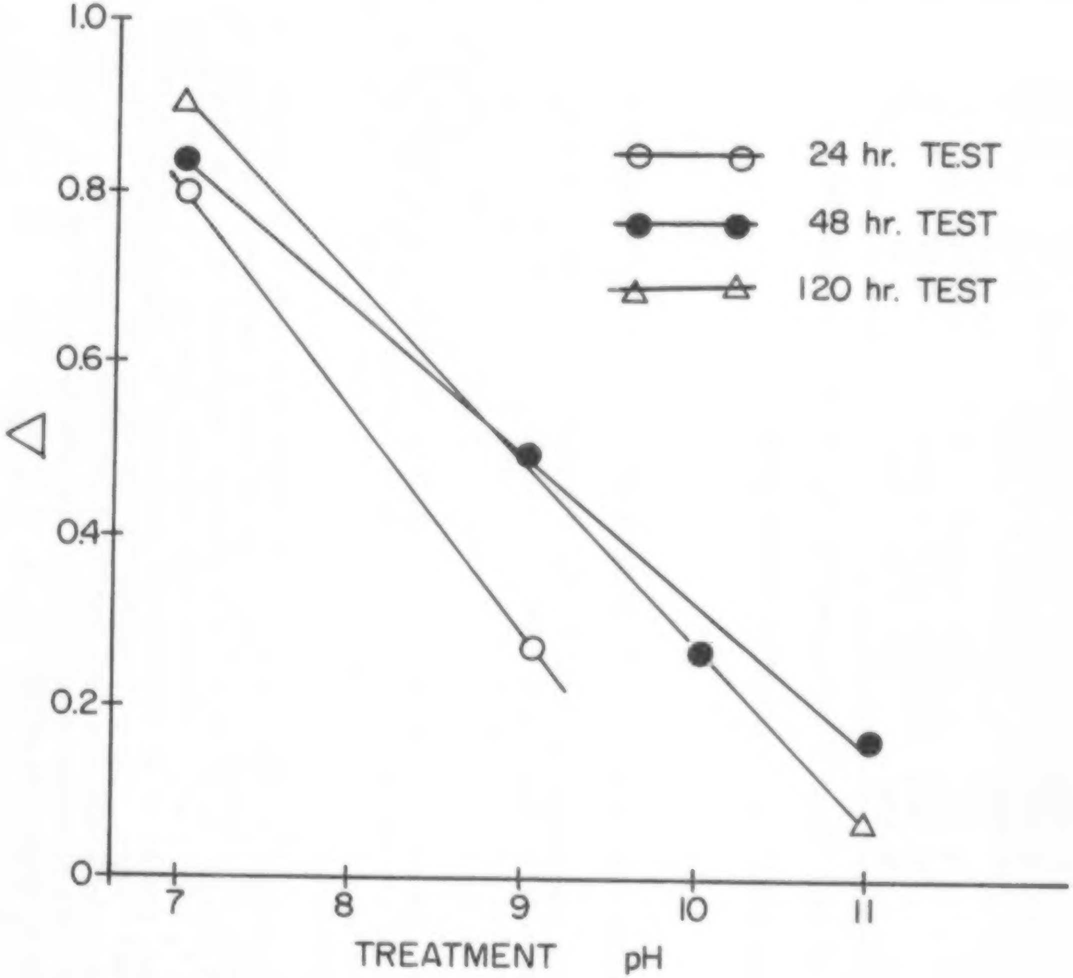


Figure 12. Detoxification (Δ) of spent chlorination liquors by alkaline treatment.

TABLE 36. EFFECT OF PROLONGED (TWO WEEK) NEUTRALIZATION ON THE TOXICITY OF SPENT CHLORINATION LIQUOR TO *DAPHNIA MAGNA*

Sample	Exposure period	EC_{50} (95% confidence interval) in ppm TOC	Δ[a]	g
SCL, pH 2	24 hrs.	172 (92.8 - 321)	1.00	0.620
	48 hrs.	70.4 (56.7 - 87.4)	1.00	0.149
	96 hrs.	55.3 (48.3 - 63.3)	1.00	0.185
SCL, pH 7	24 hrs.	>218 {20%}[b]	<0.71	-
	48 hrs.	229 (131 - 399)	0.31	0.721
	96 hrs.	138 (91 - 211)	0.40	0.378

[a] LC_{50} of pH 2 liquor ÷ corresponding LC_{50} of pH 7 treated liquor.

[b] Percent mortality at specified concentration.

BIOLOGICAL TREATMENT OF SPENT CHLORINATION AND CAUSTIC EXTRACTION LIQUORS

The microorganisms used in the treatments consisted of a mixed microbial population (hereafter referred to as "seed") contained in a sludge from a commercial pulp and paper waste treatment facility and acclimated to bleaching wastes and of pure cultures of *Candida utilis* (yeast) and an unidentified bacterium. The latter microorganism was isolated from the sludge referred to above. The biological treatment conditions are described in the Materials and Methods section. Following one-week treatment periods, the liquors were subjected to acute toxicity bioassay and the results obtained are reported in Table 37.

Treatment of spent chlorination liquor with seed in the presence of phosphate and asparagine (tests 4 and 6, resp.) resulted in complete detoxification. Surprisingly, the controls corresponding to the above (tests 3 and 5, resp.) which differed from the test samples only by the absence of seed, proved to be considerably more toxic to *Daphnia magna* than the original liquor. The explanation for the increased toxicity of the control is not immediately apparent, but in an independent experiment, it was established that a phosphate solution of the same concentration was, by itself, non-toxic to *Daphnia magna*.

In the case of spent caustic extraction liquor, the addition of phosphate in the absence of seed produced a liquor having a toxicity little different from that of the original whereas a substantial toxicity reduction occurred in the presence of seed (tests 7 and 8, resp., Table 37). When asparagine was added as a nutrient, the toxicity of the control sample (test 9) once again surpassed that of the original liquor. In the presence of seed, however, the toxicity of the liquor was reduced slightly as compared to the value for the original liquor (cf. tests 2 and 10).

TABLE 37. EFFECT OF BIOLOGICAL TREATMENTS OF SCL AND SCEL ON ACUTE TOXICITY

Test No.	Sample Description	Test Period, hrs.	LC_{50} (95% C.I.)	
			% (v/v)	ppm of TOC
1	Original SCL	24	43.4 (55.3-33.6)	102 (130-79)
		48	33.2 (45.5-21.7)	78 (107-51)
2	Original SCEL	24	37.3 (48.3-29.0)	501 (648-389)
		48	27.6	371
	Seed from sludge acclimated to bleach plant wastes			
3	SCL - Control; 5.7 x 10^{-4} M in K_2HPO_4	24	<13 (-)^{a}	<23 (-)^{a}
		48	<13 (-)^{a}	<23 (-)^{a}
4	SCL - Test; 5.7 x 10^{-4} M in K_2HPO_4 + seed	24	>100 (-)^{b}	>114 (-)^{b}
		48	>100 (-)^{b}	>114 (-)^{b}
5	SCL - Control; 7.4 x 10^{-5} M in Asparagine	24	16.9 (22.4 - 12.8)	-^{c}
		48	15.6 (20.7 - 11.8)	-^{c}
6	SCL - Test; 7.4 x 10^{-5} M in KH_2PO_4; 15 x 10^{-3} M in asparagine + seed	24	>100 (-)^{b}	-^{c}
		48	>100 (-)^{b}	-^{c}
7	SCEL - Control; 7.4 x 10^{-4} M in KH_2PO_4	24	45.2	542
		48	45.2	542
8	SCEL - Test; 7.4 x 10^{-4} M in KH_2PO_4 + seed	24	74.5 (96.3-57.7)	708 (915-548)
		48	46.9 (57.2-38.4)	446 (543-365)
9	SCEL - Control; 5.7 x 10^{-5} M in KH_2PO_4; 1.5 x 10^{-3} M in asparagine	24	9.2 (-)^{a,d}	-^{c}
		48	9.2 (-)^{a}	-^{c}
10	SCEL - Test; 5.7 x 10^{-5} M in KH_2PO_4; 1.5 x 10^{-3} M in asparagine + seed	24	52.7 (62.0-44.8)	-^{c}
		48	48.6 (59.3-39.8)	-^{c}

(continued)

TABLE 37 (continued)

Test o.		Sample description	Test period, hrs.	LC_{50} (95% C.I.), %
11	Candida utilis (yeast)	SCL - Control; 7.4×10^{-4} M in K_2HPO_4 and 5.6×10^{-3} M in glucose	24	<13 (-)[a]
			48	<13 (-)[a]
12		SCL - Test; 7.4×10^{-4} M in K_2HPO_4 and 5.6×10^{-3} M in glucose + yeast	24	>100 (-)[d]
			48	< 20 (-)[a]
13		SCEL - Control; 5.7 M x 10^{-4} M in KH_2PO_4 and 5.6×10^{-3} M in glucose	24	55.1
			48	36.7
14		SCEL - Test; 5.7 M x 10^{-4} M in KH_2PO_4 and 5.6×10^{-3} M in Glucose + yeast	24	80.8 (101.6-64.2)
			48	33.8 (34.8-28.7)
15	A Bacterium (isolate B-2)	SCL - Control; 7.4×10^{-4} M in K_2HPO_4 and 5.6×10^{-3} M in glucose	24	4.4
			48	<3.1 (-)[a]
16		SCL - Test; 7.4×10^{-4} M in K_2HPO_4 and 5.6×10^{-3} M in glucose + bacterium	24	>13 (too erratic to determine)
			48	<13 (-)[a]
17		SCEL - Control; 5.7 M x 10^{-4} M in KH_2PO_4 and 5.6×10^{-3} M in glucose	24	38.6 (49.6-30.1)
			48	25.3 (31.0-20.6)
18		SCEL - Test; 5.7×10^{-4} M in KH_2PO_4 and 5.6×10^{-3} M in glucose + bacterium	24	47.3
			48	30.0

[a] Mortality too high to calculate.

[b] Mortality too low to calculate.

[c] Incalculable because carbon source (asparagine) was added as nutrient

[d] Erratic death pattern.

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Since neither asparagine, which serves as a nutrient, nor phosphate, which functions both as a buffer and a nutrient, are required for adequate growth of the microorganisms comprising the seed, their elimination individually and in combination in a repetition of the aforegoing tests could help in identifying the cause of the high toxicity often found in the control tests.

When properly performed, biological treatment generally has been effective in reducing or eliminating the toxicity of bleached kraft mill wastes (59-63). In this connection, the available toxicity data, as can best be determined, applies exclusively to the combined chlorination and caustic extraction effluent rather than to individual bleach streams, and thus represents the composite property. The results in Table 37 indicate that biological treatment of the segregated streams (SCL and SCEL in this case) does not necessarily effect full or equivalent toxicity reduction since, in this particular treatment, SCL was detoxified to a greater extent than SCEL.

Unlike the previous situation, involving a mixed microbial population ("seed") from a sludge, an external nutrient source (glucose was used) was required to produce a respectable amount of microorganism growth in the cases of the experiments with *Candida utilis* (yeast) and the bacterium. With respect to the former microorganism, SCL was successfully detoxified in the presence of phosphate and glucose based on the result from the 24-hour test. After 48-hour exposure to the liquor, however, the *Daphnia magna* displayed an exceptionally high incidence of death (test 12). As in other previous instances, the control sample was considerably more toxic than the untreated liquor (cf. tests 1 and 11).

The results from the treatment of SCEL with yeast in regard to degree of detoxification generally paralleled those obtained when a mixed population of microorganisms (seed) was applied (compare tests 13 and 14 with 7 and 8, Table 37).

In addition to its potential utility as a microorganism capable of effecting the biodegradation of SCL and SCEL, *Candida utilis* was also selected by reason of its being an edible protein source. Therefore, to the extent that it would be able to use SCL and SCEL as carbon sources for protein production, this fungus offered some interesting possibilities. Unfortunately, the carbon concentration provided by the organic solids in the two liquors was insufficient to provide the amount of growth required to make such a process fully practicable. Thus, although the future of *Candida utilis* does not appear to be particularly bright with respect to the application described above, additional work should be performed before this approach is abandoned.

Generally speaking, the bacterium was ineffectual in reducing the toxicity of either SCL or SCEL (tests 15-18, Table 37), and the death pattern of the *Daphnia magna* was very erratic in the tests employing the former liquor.

REDUCTION IN SCL AND SCEL TOXICITY THROUGH MODIFICATIONS OF THE BLEACHING PROCESS

An alternative to the approach of eliminating bleaching liquor toxicity by chemical or biological treatment of the bleaching effluent is to modify conventional bleaching stages in such a way that the offending chemicals are either not produced or are formed in smaller amounts. The latter approach has been adopted by several investigators for the purpose of achieving not only toxicity reduction but color, COD, and BOD improvements as well. Notable in this connection are modifications involving the partial replacement of chlorine by chlorine dioxide in the first stage (57, 64, 65), replacement of chlorine with hypochlorite (66), substitution of the first caustic extraction stage by a hypochlorination treatment (64), and the addition of hydrogen peroxide to caustic extraction stages (67). In another investigation (65), novel bleaching sequences involving combinations of gaseous chlorine and chlorine dioxide, oxygen and hydrogen peroxide have been explored as possible means of reducing effluent toxicity.

In the present investigation, several combinations of the modification treatments cited above were evaluated for their effect on toxicity. Except as otherwise noted, the pulp used in the bleaching tests was a commercial sample of southern pine kraft taken from the same batch used for the preparation of the spent liquors. The "conventional" and process-modified liquors were subjected to acute toxicity bioassays using Daphnia magna as the test organism. The test conditions and LC_{50} values at various periods of exposure appear in Table 38. In the case of the 24-hour tests, the results are also expressed as toxic units.

When the caustic extraction stage was replaced by hypochlorite and alkaline hydrogen peroxide treatments (Table 38, tests 3 and 4, resp.), the acute toxicity was not substantially different from that found when a conventional extraction stage was employed. The finding with respect to hypochlorite treatment is contrary to that reported by Gall and Thompson (64); who determined that introduction of hypochlorite into the second stage extraction had the effect of reducing the acute toxicity of the resulting effluent.

Sequential ClO_2/Cl_2 treatment (Table 38, test 5) caused a pronounced reduction in effluent toxicity as compared to the situation where an equivalent amount of chlorine alone was applied (test 1). Subsequent caustic extraction of the ClO_2/Cl_2-treated pulp produced an effluent with a somewhat lower toxicity than that resulting from caustic extraction of the pulp reacted with chlorine alone (cf. tests 2 and 6). The introduction of peroxide into the extraction stage of the pulp from a ClO_2/Cl_2 sequential treatment also proved advantageous. On the other hand, the use of an equivalent amount of hypochlorite in the caustic extraction of the same pulp at the very best produced no advantage and may even have had a detrimental effect with respect to acute toxicity (cf. tests 2 and 8).

TABLE 38. TOXICITY OF EFFLUENTS FROM CONVENTIONAL AND PROCESS-MODIFIED BLEACHING STAGES

Test no.	Treatment or treatment sequence[a]	LC_{50}, ppm TOC[b]				24-hr toxic units ($TOC/24-LC_{50}$ TOC)
		24-hr	48-hr	72-hr	96-hr	
1	C	94	51	-	-	2.4
2	C,E	480	410	380	-	2.9
3	C,H	430	280	220	160	3.0
4	C,P	524	467	-	-	2.9
5	D → C	186	55	-	-	1.4
6	D → C,E	660	421	-	-	2.3
7	D → C,P	789	476	-	-	1.8
8	D → C,H	350	190	-	-	3.6
9	D	380	150	100	70	0.6
10	D,E	600	423	340	310	2.0
11	D,H	430	265	180	140	2.6
12	A/O	560	400	350	280	-

[a] C = chlorine (5.25%); E = caustic extraction (3.6% NaOH); P = hydrogen peroxide (0.5%); H = sodium hypochlorite (2.0%); D = chlorine dioxide (2.0%); D → C = sequential chlorine dioxide (1.0% ClO_2); A/O = alkaline-oxygen (80 psig oxygen).

[b] Values corresponding to second-stage effluents in two-stage treatments.

When the first bleaching stage consisted of the sole application of chlorine dioxide (test 9), toxicity reduction was further enhanced as compared to the cases where an equivalent amount of chlorine was used alone or sequentially in combination with chlorine dioxide (tests 1 and 5, resp.). Subsequent extraction of the dioxide-treated pulp with alkali (test 10) generated a liquor having an acute toxicity less than that shown by the caustic extraction liquor derived from the chlorinated pulp (cf. tests 10 and 2) but in the same range as the liquor obtained by extracting the ClO_2/Cl_2-treated pulp. Extraction of the dioxide-treated pulp with hypochlorite (test 11) appeared, on the other hand, to enhance slightly the toxicity of the resulting liquor as compared to the case where a conventional alkali extraction was employed (cf. tests 10 and 11).

The final test recorded in Table 38 (test 12) was performed in an industrial laboratory on a sample of southern pine kraft (Kappa No. 39.2) and a portion of the spent liquor was provided for acute toxicity bioassay. The effectiveness of the alkali/oxygen treatment is probably best judged by comparison with the toxicity of other alkaline spent liquors listed in Table 38. Such a comparison leads to the conclusion that the acute toxicity level, in this case,is in the same range as those for the other alkaline liquors listed in the table with the exception of the situations where hypochlorite was employed. However, the advantage of the A/O treatment lies in the fact that it requires no prior bleaching stage as the other modification sequences in Table 38 do, thus eliminating a source of material having a relatively high toxicity.

Since the foregoing bioassay results indicated the value of replacing all or part of the chlorine by chlorine dioxide in the first stage as a means of reducing the acute toxicity of first- and second-stage effluents, these spent liquors were subjected to additional characterization tests in an effort to understand the reason for this effect. The results of these analyses are shown in Table 39.

The partial or total replacement of chlorine by chlorine dioxide is seen not to have had a particularly significant effect on either the color or TOC levels of the first-stage effluents. On the other hand, the color of the effluents from the subsequent caustic extraction treatments decreased significantly with increasing substitution of chlorine dioxide for chlorine in the first stage. The TOC contents of the extraction liquors decreased significantly only as a result of a 100% replacement of chlorine by chlorine dioxide in the first stage.

As expected, the chloride ion concentrations of all the liquors included in Table 39 decreased as the proportion of chlorine dioxide used in the first stage increased. Because of the practice followed in this investigation of washing chlorine- and/or chlorine dioxide-treated pulps with dilute hydrochloric acid rather than with tap water, the chloride ion contents of the caustic extraction liquors corresponding to pulp washed in this manner are somewhat higher than normal. The magnitude of this difference can be judged by comparing the final two values under the heading "chloride" in Table 39.

TABLE 39. CHARACTERISTICS OF SELECTED CONVENTIONAL AND PROCESS-MODIFIED SPENT BLEACHING LIQUORS

Treatment or treatment sequence[a]	Color (pt-co units)[b]	TOC (ppm)[b]	Chloride (ppm)[b]	Org.-bound chlorine, ppm[b]	$\frac{\text{Org. chlorine}}{\text{TOC}}$	$\Delta a^{b,c}$	$\frac{\Delta a}{\text{TOC}} \times 10^{-3}$
C	1290	228	973	90	0.394	0.53	2.3
D → C	1360	260	600	97	0.373	0.61	2.3
D	1120	226	179	107	0.474	0.39	1.7
C,E	17,930	1410	840	210	0.148	0.28	0.20
D → C,E	13,100	1515	380	124	0.082	0.23	0.15
D,E	7190	1220	105	5	0.004	0.10	0.08
D[d],E	-	-	45	10	-	-	-

[a] C = chlorine; D = chlorine dioxide; E = caustic extraction; D → C = sequential chlorine dioxide-chlorine treatment.

[b] Values correspond to second-stage effluents in two-stage treatments.

[c] Measurement at 290 nm for acidic liquors and 315 nm for alkaline liquors.

[d] Dioxide-treated pulp washed with 0.01 N H_2SO_4.

As expected, the organically bound chlorine contents (Table 39) of the first-stage liquors were considerably higher than those of the corresponding caustic extraction liquors. The organically bound chlorine content of the latter decreased sharply as chlorine was replaced by chlorine dioxide, and at 100% replacement, the organically bound chlorine content of the liquor was essentially nil. This finding is in general agreement with results reported by Rapson and Anderson (68). In the case of the first-stage liquors, the trend is not as clear cut, but the organically bound chlorine content of the liquor from the 100% dioxide treatment is substantially higher than for the liquors from the C and D → C stages. Inasmuch as the toxicity of the D stage effluent was the lowest of the first-stage liquors (see Table 38), the expected direct relationship between organically bound chlorine and toxicity was not demonstrated. However, the organically bound chlorine analysis includes both aliphatic- and aromatic-bound chlorine, and it is the latter which is generally thought to be the more important contributor to toxicity.

The Δ-absorbance (Δa) values in Table 39 are assumed to represent principally changes in the phenolic content of the liquors. The problems associated with the use of ionization difference spectroscopy for such a purpose have been discussed previously, and it suffices to emphasize here that the measured changes in Δa values at best merely reflect possible changes in phenol content. With this in mind, the Δa data in Table 39 reveal that a significant decrease in "phenol" content was effected only in the case where the chlorine was completely replaced by chlorine dioxide. The corresponding caustic extraction liquor showed a systematic decrease in "phenol" content with increasing replacement of chlorine by dioxide in the first stage, and this change paralleled the decrease in organically bound chlorine content of the same liquors. In none of the tests performed in this investigation was the "phenol" content reduced to zero, and it may be speculated that such a condition could not be achieved without a prohibitive expenditure of chemical.

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APPENDIX A

Jack Meyer's Modified Medium for Chlorella

Stock Solutions	ml/l
1 M KNO_3	12
1 M $MgSO_4 \cdot 7H_2O$	10
1 M KH_2PO_4	9
Fe Versenol[a]	1
Micronutrients[b]	1
1 M $Ca(NO_3)_2$	0.1
$NaHCO_3$ (15 g/l)	1
Double distilled water	966
	1000 ml

[a] Fe Versenol: Dissolve 26.1 g Versenol in 276 ml 1 M KOH. Add double distilled water to 500 ml. Dissolve 24.9 g $FeSO_4 \cdot 7H_2O$ in 500 ml double distilled water. Mix the two solutions stirring rapidly and aerate overnight. Store in a foil covered container under refrigeration because the rotation is light and temperature sensitive.

[b] Micronutrients:

Ingredients	Conc. in Stock Solution g/l
$CoCl_2 \cdot 6H_2O$	0.040
HBO_3	2.86
$MnCl_2 \cdot 4\ H_2O$	1.81
$ZnSO_4 \cdot 7\ H_2O$	0.222
$CuSO_4 \cdot 5\ H_2O$	0.079
MoO_3 (97.5%)	0.015

APPENDIX B

Hutner's Medium for Duckweed

Solution 1: 17.7 g of $Ca(NO_3)_2 \cdot 4H_2O$ and 10.0 g of NH_4NO_3/liter

Solution 2: 20 g of K_2HPO_4/l

Solution 3A: 3.295 g $ZnSO_4 \cdot 7H_2O$
0.710 g H_3BO_3
1.260 g $Na_2\ MoO_4 \cdot 2H_2O$
0.190 g $CuSO_4 \cdot 5H_2O$
Add HCl to disperse cloudiness
0.010 g $Co(NO_3)_2$
0.897 $MnCl_2 \cdot 4H_2O$
dissolved in 200 ml of water

Solution 3B: 25.0g of EDTA, add sufficient KOH to dissolve and ~400 ml water

Solution 3C: 1.25 g $FeSO_4 \cdot 7H_2O$ dissolved in ~ 200 ml water

Solution C was prepared by adding 3B to 3C and then adding 3A. The solution was diluted to one liter and the pH adjusted to 6.

Solution 4: 25.0 g of $MgSO_4 \cdot 7HO$/l

Hutner's medium (1/2 strength) consists of 5 ml each of solutions 1-4 and 580 ml of distilled H_2O. Ten g of glucose is added, the pH adjusted to 6.1-6.4 and the solution is autoclaved.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/2-80-039	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Toxicity Reduction Through Chemical And Biological Modification of Spent Pulp Bleaching Liquors		5. REPORT DATE January 1980 issuing date 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Carlton Dence, Chun-Juan Wang, and Patrick Durkin		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS State University of New York College of Environmental Science And Forestry Syracuse, N.Y. 13210		10. PROGRAM ELEMENT NO. 1BB610 11. CONTRACT/GRANT NO. R-804779
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15. SUPPLEMENTARY NOTES

16. ABSTRACT

Chlorophenols similar to or identical with those detected in spent chlorination and caustic extraction liquors were synthesized and tested over a range of concentrations to determine their effect on the growth of several fungi, an alga (Chlorella pyrenoidosa) and duckweed (Lemna perpusilla) and on the survival of Daphniamagna.

Biological treatment of the chlorophenols consisted of the application of pure cultures of three different fungi and a mixed microbial population for periods ranging up to 15 days. Degradation varied widely among the various phenols and for the same phenol treated with different fungi.

Spent chlorination and caustic extraction liquors were subjected to a variety of chemical treatments and the resulting effects on acute toxicity determined. Treatment with elemental chlorine, hypochlorous acid, hypochlorite, ozone and hydrogen peroxide produced increases in the toxicity of the spent liquor.

Biological treatment of spent chlorination and caustic extraction liquors involved the application of a fungus (Candida utilis), an unidentified bacterium, and a mixed microbial population, together with supplemental carbon sources.

Toxicity reduction through modification of conventional chlorination and caustic extraction bleaching stages was also evaluated.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pulping, Industrial Wastes, Phenols, Bioassay, chemical treatment, toxicity, biological treatment	Spent Chlorination liquors, chlorophenols, Total organic carbon	13/B

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